DETECTION OF RENAL DYSFUNCTION DURING AND AFTER ANESTHESIA AND SURGERY: EVALUATION OF THE INFLUENCE OF INORGANIC FLUORIDE, KETOROLAC AND CLONIDINE

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Helsinki 2006

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ACADEMIC DISSERTATION

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ABSTRACT

Drugs and surgical techniques may have harmful renal effects during the perioperative period. Traditional biomarkers are often insensitive to minor renal changes, but novel biomarkers may more accurately detect disturbances in glomerular and tubular function and integrity. The purpose of this study was first, to evaluate the renal effects of ketorolac and clonidine during inhalation anesthesia with sevoflurane and isoflurane, and secondly, to evaluate the effect of tobacco smoking on the production of inorganic fluoride (F^-) following enflurane and sevoflurane anesthesia as well as to determine the effect of F^- on renal function and cellular integrity in surgical patients.

A total of 143 patients undergoing either conventional (n = 75) or endoscopic (n = 68) inpatient surgery were enrolled in four studies. The ketorolac and clonidine studies were prospective, randomized, placebo controlled and double-blinded, while the cigarette smoking studies were prospective cohort studies with two parallel groups.

As a sign of proximal tubular deterioration, a similar transient increase in urine N-acetyl-β-D-glucosaminidase/creatinine (U-NAG/crea) was noted in both the ketorolac group and in the controls (baseline vs. at two hours of anesthesia, p = 0.015) with a 3.3 minimum alveolar concentration hour sevoflurane anesthesia. Uncorrelated U-NAG increased above the maximum concentration measured from healthy volunteers (6.1 units L^{-1}) in 5/15 patients with ketorolac and in none of the controls (p = 0.042). As a sign of proximal tubular deterioration, U-glutathione transferase-α/crea (U-GST-α/crea) increased in both groups at two hours after anesthesia but a more significant increase was noted in the patients with ketorolac. U-GST-α/crea increased above the maximum ratio measured from healthy volunteers in 7/15 patients with ketorolac and in 3/15 controls.

Clonidine diminished the activation of the renin-angiotensin aldosterone system during pneumoperitoneum; urine output was better preserved in the patients treated with clonidine (1/15 patients developed oliguria) than in the controls (8/15 developed oliguria (p=0.005)). Most patients with pneumoperitoneum and isoflurane anesthesia developed a transient proximal tubular deterioration, as U-NAG increased above 6.1 units L^{-1} in 11/15 patients with clonidine and in 7/15 controls. In the patients receiving clonidine treatment, the median of U-NAG/crea was higher than in the controls at 60 minutes of pneumoperitoneum (p = 0.01), suggesting that clonidine seems to worsen proximal tubular deterioration.

Smoking induced the metabolism of enflurane, but the renal function remained intact in both the smokers and the non-smokers with enflurane anesthesia. On the contrary, smoking did not induce sevoflurane metabolism, but glomerular function decreased in 4/25 non-smokers and in 7/25 smokers with sevoflurane anesthesia. All five patients with S-F^- ≥ 40 μmol L^{-1}, but only 6/45 with S-F^- less than 40 μmol L^{-1} (p = 0.001), developed a S-tumor associated trypsin inhibitor concentration above 3 nmol L^{-1} as a sign of glomerular dysfunction. As a sign of proximal tubular deterioration, U-β2-microglobulin increased in 2/5 patients with S-F^- over 40 μmol L^{-1} compared to 2/45 patients with the highest S-F^- less than 40 μmol L^{-1} (p = 0.005).

To conclude, sevoflurane anesthesia may cause a transient proximal tubular deterioration which may be worsened by a co-administration of ketorolac. Clonidine premedication prevents the activation of the renin-angiotensin aldosterone system and preserves normal urine output, but may be harmful for proximal tubules during pneumoperitoneum. Smoking induces the me-

tabolism of enflurane but not that of sevoflurane. Serum F^- of 40 μmol L^{-1} or higher may induce glomerular dysfunction and proximal tubular deterioration in patients with sevoflurane anesthesia. The novel renal biomarkers warrant further studies in order to establish reference values for surgical patients having inhalation anesthesia.

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ABBREVIATIONS

ADH	antidiuretic hormone
α1-MG	α1-microglobulin
ASA	American Society of Anesthesiologists physical status
AUC	area under the time concentration curve
ARF	acute renal failure
β2-MG	β2-microglobulin
CI	confidence interval
CO_2	carbon dioxide
COX	cyclo-oxygenase
crea	creatinine
CYP	cytochrome P 450
F^-	inorganic fluoride
GST-α	glutathione-S-transferase-α
GST-π	glutathione-S-transferase-π
GFR	glomerular filtration rate
IAP	intra-abdominal pressure
i.m.	intramuscular
i.v.	intravenous
M	molar mass
MAC	minimum alveolar concentration
MAC-hour	minimum alveolar concentration-hour
NAG	N-acetyl-β-D-glucosaminidase
NSAID	nonsteroidal anti-inflammatory analgesic drug
PG	prostaglandin
P	plasma
RAAS	renin-angiotensin-aldosterone system
RA	renin activity
RBF	renal blood flow
S	serum
TATI	tumor associated trypsin inhibitor
U	urine

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which will be referred to in the text with the Roman numerals I to V. Some unpublished data is also presented.

I Laisalmi M, Eriksson H, Koivusalo A-M, Pere P, Rosenberg P, Lindgren L. Ketorolac is not nephrotoxic in connection with sevoflurane anesthesia in patients undergoing breast surgery. Anesthesia and Analgesia 2001; 92(4): 1058–1068.

II Laisalmi M, Teppo A-M, Koivusalo A-M, Honkanen E, Valta P, Lindgren L. Effect of ketorolac and sevoflurane anesthesia on renal glomerular and tubular function. Anesthesia and Analgesia 2001; 93(5): 1210–1213.

III Laisalmi M, Koivusalo A-M, Valta P, Tikkanen I, and Lindgren L. Clonidine provides opioid-sparing effect, stable hemodynamics, and renal integrity during laparoscopic cholecystectomy. Surgical Endoscopy 2001; 15(11): 1331–1335.

IV Laisalmi M, Soikkeli A, Kokki H, Markkanen H, Yli-Hankala A, Rosenberg P, Lindgren L. Fluoride metabolism in smokers and non-smokers following enflurane anaesthesia. British Journal of Anaesthesia 2003; 91(6): 800–804.

V Laisalmi M, Soikkeli A, Kokki H, Markkanen H, Yli-Hankala A, Rosenberg P, Lindgren L. Effects of cigarette smoking on serum fluoride concentrations and renal function markers after sevoflurane anaesthesia. Acta Anaesthesiologica Scandinavica 2006; 50(8): 982–987.

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INTRODUCTION

The kidneys' feedback systems are important for maintaining body homeostasis. The kidneys adjust the water, electrolyte, and acid-base balance, and remove waste products of metabolism. In addition, the kidneys regulate endocrine functions, such as erythropoiesis and renin-angiotensin-aldosterone system (RAAS) (Ganong 2003).

In Finland, 3580 patients i.e. 684 per million adults received renal replacement therapy in 2004 (Finnish Registry of Kidney Disease 2004). Although severe renal deterioration is a rare condition, acute renal failure (ARF) developing during surgery and leading to renal replacement therapy is an independent risk factor for mortality (Chertow et al. 1998).

In clinical practice urine output, serum creatinine (S-crea) and S-urea are used to assess renal function. However, these methods are insensitive to minor changes in renal function; almost half of renal glomerular function is lost when S-crea and S-urea increase (Kellen et al. 1994, Star 1998). Thus, more specific and sensitive markers are needed.

Several biomarkers are released into the serum and urine when kidney function and cellular integrity is deteriorated. Glomerular function may be assessed using S-cystatin C and S-tumor associated trypsin inhibitor (S-TATI) (Tramonti et al. 1997, Harmoinen 2001). Proximal tubular dysfunction may be assessed with U-α1-microglobulin/creatinine (U-α1-MG/crea) (Teppo et al. 2000) and cellular integrity with U-N-acetyl-β-D-glucosaminidase/creatinine (U-NAG/crea) (Higuchi et al. 1995) and U-glutathione transferase-α (U-GST-α) ((Branten et al. 2000). Urine glutathione transferase-π (U-GST-π) may be used to determine the distal tubular cellular integrity (Usuda et al. 1999). However, these markers are primarily used in research and not in clinical practice.

Metabolism of anesthetic drugs produces substances, which may be toxic to the kidneys (Taves et al. 1970). Inorganic fluoride (F^-) is released during the metabolism of halogenated inhalation anesthetics, e.g. enflurane and sevoflurane (Kharasch et al. 1994, Kharasch 1995). A prolonged anesthesia with enflurane causes disturbances of renal concentrating ability and decreases glomerular filtration rate (GFR) (Mazze et al. 1977). After prolonged sevoflurane anesthesia, high levels of $S\text{-}F^-$ can be found and renal proximal tubular damage markers are released into the urine (Higuchi et al. 1995, Eger et al. 1997), although conflicting results have been published (Ebert et al. 1998a).

Other factors, however, related to the patient, anesthesia and surgery may alter the risk for renal failure. Cigarette smoking is common among Finnish adults (Uutela and Koskinen 2002). Enzyme induction or inhibition by cigarette smoke may lead to altered metabolism of drugs (Vähäkangas et al. 1983, Miller 1990). Enflurane and sevoflurane are metabolized in the liver by the enzyme, cytochrome P450 2E1 (CYP 2E1), (Kharasch and Thummel 1993, Kharasch et al. 1994) and tobacco smoke consists of active substances that inhibit CYP 2E1, e.g. nicotine and its metabolite cotine (Van Vleet et al. 2001). However, whether smoking affects enflurane and sevoflurane metabolism remains unknown.

In patients with decreased circulating blood volume, non–steroidal anti-inflammatory drugs (NSAIDs) may impair renal function. A deficit in intravascular volume leads to the release of catecholamines and the synthesis of angiotensin II, which may induce renal vasoconstriction (Lameire et al. 2005). By preventing the synthesis of vasodilating prostaglandins (PGs) in the

kidneys, NSAIDs predispose the kidneys to vasoconstriction and ischemia (Thadhani et al. 1996).

Clonidine, an α_2-adrenergic receptor agonist, decreases the need of anesthetics and opioids during surgery (Engelman et al. 1989, De Deyne et al. 2000). Clonidine causes vasodilatation in renal arteries and may inhibit the effect of antidiuretic hormone (ADH) on collecting tubules (Reid et al. 1979) and prevent the increase in serum noradrenaline concentration (Joris et al. 1998). During surgery, clonidine preserves normal urine output (Hamaya et al. 1994).

Laparoscopic surgery is increasingly used for abdominal surgery. The surgical visual field is created by insufflating carbon dioxide (CO_2) into the peritoneal cavity. Pneumoperitoneum causes a release of catecholamines (Joris et al. 1998), activates RAAS (Lindgren et al. 1995), increases ADH secretion (Viinamäki and Punnonen 1982) and compresses the kidney and renal vasculature (Doty et al. 2000). All these factors may induce renal ischemia and cause proximal tubular damage and diminished urine output.

This study evaluated the effects of different factors on renal function during the perioperative period using novel and sensitive biomarkers of renal function and cellular integrity. We first examined the renal effects of ketorolac during sevoflurane anesthesia; next, we examined the renal effects of clonidine during laparoscopic cholecystectomy; and thirdly, we studied the effects of smoking on production of F^- after enflurane and sevoflurane anesthesia, and the renal effects of the released F^-.

REVIEW OF THE LITERATURE

KIDNEY AND RENAL FUNCTION

The filtering capillary network of a nephron is called a glomerulus. A nephron, numbering one million in each kidney, consists of a glomerulus and a tubular system. The glomerulus is situated between an afferent and an efferent arteriole. The nephrons are divided into cortical and medullary nephrons, according to their position, length of Henle's loops, and vascular supply. The majority of nephrons are situated in the cortical region gaining their blood supply from glomerular efferent arterioles. One seventh of the nephrons and their loop's of Henle are in the medullary region and the arcuate artery (arteria arcuata) is the source of their blood supply.

The glomerular filtrate passes to the proximal tubule. From there the filtrate passes into the loop of Henle, which dives towards the cortical or medullary region. The loop of Henle consists of two segments, the descending and ascending loops. The ascending loop is divided into two different parts, an initial thin segment that turns into a thick segment.

The distal part of the tubule begins after the loop of Henle, near the cortical region. At the cortical region, the distal tubules join to form a collecting duct, entering the medullary area and coalescing to the renal pelvis (Tischer and Madsen 2004). The juxtaglomerular apparatus, situated in proximity of the distal tubule, consists of granular cells, which synthesize and release renin. Renin acts with the angiotensin converting enzyme to produce angiotensin II from angiotensinogen. Angiotensin II induces arteriolar vasoconstriction and aldosterone secretion in the adrenal cortex and ADH secretion in the pituitary gland. The RAAS increases blood pressure, decreases glomerular filtration rate (GFR) and increases sodium and water reabsorption in the collecting ducts (Ganong 2003).

Blood to be filtered enters the glomerulus via afferent and leaves via efferent arterioles. Blood for the tubular network comes from the glomerular efferent arterioles or arcuate arteries and follows the loops of Henle supplying deep medullary regions as vasa recta vessels (arteriolae and venae rectae). From there the blood enters the venous system (Ganong 2003).

Renal blood flow (RBF) varies in the different regions of the kidney; in the cortical region, blood flow is 4 mL min^{-1} g^{-1} of renal tissue and in the outer medullary region it is 2 mL min^{-1} g^{-1} (Ganong 2003). Although the kidneys receive one fifth of cardiac output and the renal basal oxygen consumption is one tenth of the total oxygen consumption of the body, the oxygen partial pressure at the medullary vasa recta arterioles is low, approximately 1.4 kPa. Due to low oxygen partial pressure, the metabolically active proximal convoluted tubules and deep loops of Henle are vulnerable to ischemia (Kopolovic et al. 1989, Brezis et al. 1989).

Blood flow autoregulation and the tubuloglomerular feedback mechanism maintain intrarenal oxygen homeostasis (Shipley and Study 1951). The blood flow is maintained at normal levels despite large variations in blood pressure by altering the arteriolar tone (renal autoregulation). The mechanism of autoregulation is not completely understood. However, it is known that the autoregulatory response includes the action of the intrinsic renal vasoconstrictor angiotensin II, the effect of vasodilating renal PGs and the myogenic contractile response of the renal vasculature (Dibona and Sawin 2004). In the case of decreased delivery of sodium and chloride ions to distal tubules or decreased blood pressure, the tubuloglomerular feedback mechanism induces renin production and activates RAAS (Ganong 2003).

The glomerules produce 180 L glomerular filtrate (ultrafiltrate) per day. Most of glomerular filtrate is reabsorbed to the circulation and the daily urine output is one to two liters. Glomerular filtration pressure is the major determinant of GFR (Ganong 2003). Glomerular filtration pressure is affected by pressure in afferent and efferent arterioles and glomerular oncotic pressure. Intense sympathetic or angiotensin II stimulation causes vasoconstriction in afferent arterioles and a drop in GFR. A lesser sympathetic or angiotensin II stimulation increases efferent arterioles vascular tone, subsequently increasing GFR (Hall et al. 1977).

The majority of the ultrafiltrate, e.g. ions, amino acids, and organic solutes, is reabsorbed in the proximal convoluted tubule (Ganong 2003). There are several active transport systems in the proximal tubules, which reabsorb solutes from tubular fluid. Reabsorption occurs against a concentration gradient and is energy-dependent (Ganong 2003).

The hyperosmotic medulla is created by the loops of Henle, which have segmentally different permeability to water and active transport systems to sodium, chloride and urea in order to create hyperosmotic medulla. In the vasa recta vessels, the blood flow is limited to maintain medullary solutes, sodium, chloride and urea, formed by the thick ascending loops of Henle (Brezis et al. 1989, Ganong 2003).

The distal convoluted tubule is impermeable to water, although sodium and chloride are actively reabsorbed and potassium and hydrogen ions are secreted. The collecting ducts are only slightly permeable to water in the absence of ADH. When ADH is present, the collecting ducts are permeable to water so that water is reabsorbed and less urine is excreted (Ganong 2003).

ACUTE RENAL FAILURE

There is a paucity of generally accepted diagnostic criteria for ARF. The lack of diagnostic consensus makes the assessment and comparison of different study results difficult. Recently the Conference of the Acute Dialysis Quality Initiative group published the first consensus definitions and classifications of ARF. These criteria, called RIFLE (Risk of renal dysfunction, Injury to the kidney, Failure of the kidney function, Loss of kidney function and End stage renal disease), use S-crea, GFR or urine output as defining the stages of renal deterioration (Bellomo et al. 2004). The patient may fulfill the criteria through changes in GFR, S-crea or urine output (table 1).

Table 1.The RIFLE classification for assessment the stage of renal deterioration (modified from Bellomo et al 2004)

	GFR criteria	Urine output criteria
Risk of renal dysfunction	S-crea x 150% or GFR decrease > 25% from baseline	Urine output < 0.5 mL kg^{-1} h^{-1} for 6 hours
Injury to the kidney	S-crea x 200% or GFR decrease > 50%	Urine output < 0.5 mL kg^{-1} h^{-1} for 12 hours
Failure of the kidney function	S-crea x 300% or GFR decrease 75% or S-crea >176 µmol L^{-1}	Urine output < 0.3 mL kg^{-1} h^{-1} for 24 hours or anuria for 12 hours
Loss of kidney function	Persistent ARF duration > 4 weeks	
ESKD	End stage kidney disease (> 3 months)	

RIFLE = Risk of renal dysfunction, Injury to the kidney, Failure of the kidney function, Loss of kidney function and End stage renal disease, GFR = glomerular filtration rate, S-crea = serum creatinine, ARF = acute renal failure, ESKD = end stage kidney disease.

On the basis of different causal factors ARF is classified into three categories: prerenal, renal or intrinsic, and postrenal.

Prerenal ARF results from a fall in renal perfusion. Renal perfusion may decline due to hypovolemia and hypotension. When renal perfusion pressure decreases, GFR is controlled by modulating the tone in afferent and efferent arterioles. The tone in the afferent arteriole is adjusted by renal autoregulation, renal vasodilatory PGs and the tone in the efferent arteriole by angiotensin II, respectively (Brady et al. 2004). Drugs interfering with renal autoregulation, e. g. angiotensin converting-enzyme inhibitors or NSAIDs, may further decrease GFR. Usually, renal function returns to normal when perfusion is restored. However, acute cortical necrosis may ensue if poor renal perfusion persists.

Renal or intrinsic aetiology for ARF arises from various renal diseases, occlusion of renal vessels, ischemia, nephrotoxic agents, and diseases of renal microvascularity, glomeruli or tubulointerstitial processes. Many pharmacological agents and poisons may cause acute tubular necrosis by inducing intrarenal vasoconstriction, direct tubular toxicity, tubular obstruction or a combination of these (Thadhani et al. 1996).

IMPACT OF HYPOVOLEMIA ON RENAL FUNCTION

Hypovolemia leads to a drop in the mean systemic arterial pressure, which initiates activation of the sympathetic nervous system and RAAS and release of ADH. Noradrenaline, angiotensin II and ADH act to maintain blood pressure and preserve cardiac and cerebral perfusion by causing vasoconstriction in the musculocutaneous and splanchnic circulations and inhibit salt and water loss. Glomerular perfusion and GFR are preserved during mild hypoperfusion through several compensatory mechanisms. Renal autoregulation detects reduced perfusion pressure and triggers afferent arteriolar vasodilatation. Intrarenal synthesis of PGs, kallikrein and kinins is enhanced. Angiotensin II may induce efferent arteriolar vasoconstriction and intraglomerular pressure is preserved and GFR is maintained (Brady et al. 2004). High concentrations of angiotensin II provoke vasoconstriction in both afferent and efferent arterioles (Brady et al. 2004).

Under normal conditions, both the outer and inner medullary region of kidneys are at a low partial pressure of oxygen (Brezis et al. 1989). Further vasoconstriction may cause ischemia in the metabolically active segments of the proximal tubule and the medullary thick ascending limb of Henle's loop (Kopolovic et al. 1989).

In severe hypoperfusion, the renal compensatory mechanisms are overwhelmed and ARF may ensue. Renal autoregulation is maximal at a mean systemic arterial pressure of 80 mmHg, below which hypotension results in deteriorated GFR.

BIOMARKERS OF RENAL FUNCTION

TRADITIONAL BIOMARKERS

CREATININE

Creatinine (molar mass (M): 113) is formed from muscular creatine and 2% of creatine is converted to crea. Serum crea reflects GFR; as it declines, S-crea increases (Brady et al. 2004). The use of S-crea as a biomarker of renal function is based on a steady state mass balance, with assumptions that the rate of crea appearance in the blood stream is constant and balanced solely by the rate of elimination by filtration through the glomerulus (Brady et al. 2004). However, crea can also be excreted in the urine by renal tubules, and a decrease in glomerular crea filtration is compensated by an increase in crea secretion by proximal tubule cells. On the other hand, the rate of crea appearance into the blood stream depends on muscle mass (Grubb et al. 1992).

Serum crea is routinely used to measure the perioperative renal function. The major restriction for the use of S-crea is the lack of sensitivity; a 50% reduction in GFR may occur before S-crea levels increase (Renkin and Robinson 1974, Esson and Schrier 2002), and the lack of specificity; misinterpretations may be caused by dilution from fluid loading and muscle mass of the patients if left unnoticed. Thus, S-crea may detect an impairment in GFR in only a minority of patients.

CREATININE CLEARANCE

Creatinine clearance is considered a reliable measurement of GFR. Creatinine clearance measures the ability of the kidneys to clear crea from the blood into the urine over a period of time. A 24-hour urine collection should be used for accurate measurement, but shorter collection times are preferred for practical reasons. However, calculation of endogenous crea clearance requires a 24-hour urine sample collection, and non-compliance of patients and diseases, e.g. diarrhea, may adversely affect urine collection.

In clinical work the crea clearance, which is a labor-consuming technique, is not measured. For accurate measures non-compliant patients must be hospitalized during the urine collection period. Therefore, in clinical practice, glomerular function is usually estimated using calculated formulae.

Different formulae have been developed for estimating GFR from S-crea and biometric data. These formulae are based on assumptions that S-crea is constant and equal to its production, and that the production is proportional to muscle mass. Muscle mass is predicted from sex, age, weight and ethnic background. The commonly used Cockcroft-Gault formula is derived from a hospital population with only 4% of female patients (Cockcroft and Gault 1976). In a validitation study in a large population (n = 8592), the Cockcroft-Gault formula underestimated GFR and the accuracy to detect age-related renal impairment was low (Verhave et al. 2003).

The Modification of Diet in Renal Disease is a recent formula, based on data from a middle-aged population with chronic renal disease (mean S-crea 200 $\mu mol\ L^{-1}$) (Levey et al. 1999). This formula is based on age, sex, race, S-crea, S-albumin and S-urea. However, this formula has not been validated in patients without renal disease, patients with serious comorbidities or in elderly persons (Wasén 2004).

UREA

Urea (M: 60) is the product of amino acid metabolism. Urea is formed mainly in the liver and the production rate is dependent on liver function, dietary factors and nutritional status. Serum urea is filtered in the glomerulus and reabsorbed in the tubules with water. When the urine output is high, the reabsorption decreases. The decreased GFR or urine output leads to an increase in S-urea. Urea measurement is used in estimating the degree of uremia and need for dialysis therapy.

Many extrarenal factors, e.g. augmented protein intake and protein catabolic rate, affect urea production. Gastrointestinal bleeding increases S-urea and some medications, e.g. the corticosteroids or anabolic steroids, decrease S-urea (Kellen et al. 1994).

Serum urea is as insensitive marker of GFR as S-crea. Although the changes in S-crea and -urea are widely used to assess postoperative renal function, they detect less than one third of the patients with postoperative renal deterioration (Charlson et al. 1989, Kellen et al. 1994).

NOVEL BIOMARKERS

CYSTATIN C

Cystatin C, a 122-amino acid cysteine protease inhibitor (M: 13 400), is an endogenous component of plasma (Grubb and Löfberg 1982, Grubb 1992). Cystatin C is a product of a non-inducible gene that is expressed in all nucleated cells, and the cystatin C protein is stable and unmodulated (Abrahamson et al. 1990).

Cystatin C is freely filtered by the glomerulus and it is neither secreted nor reabsorbed as an intact molecule (figure 1). S-cystatin C levels better estimate GFR than S-crea (Grubb et al. 1985, Swan 1997). Serum cystatin C is considered especially useful in the diagnosis of mild renal deterioration (Bostom and Dworkin 2000). In patients with hypertension and microalbuminuria but otherwise normal GFR, S-cystatin C is elevated. This indicates that S-cystatin C is useful in detecting patients with early nephropathy with normal GFR (Coll et al. 2000).

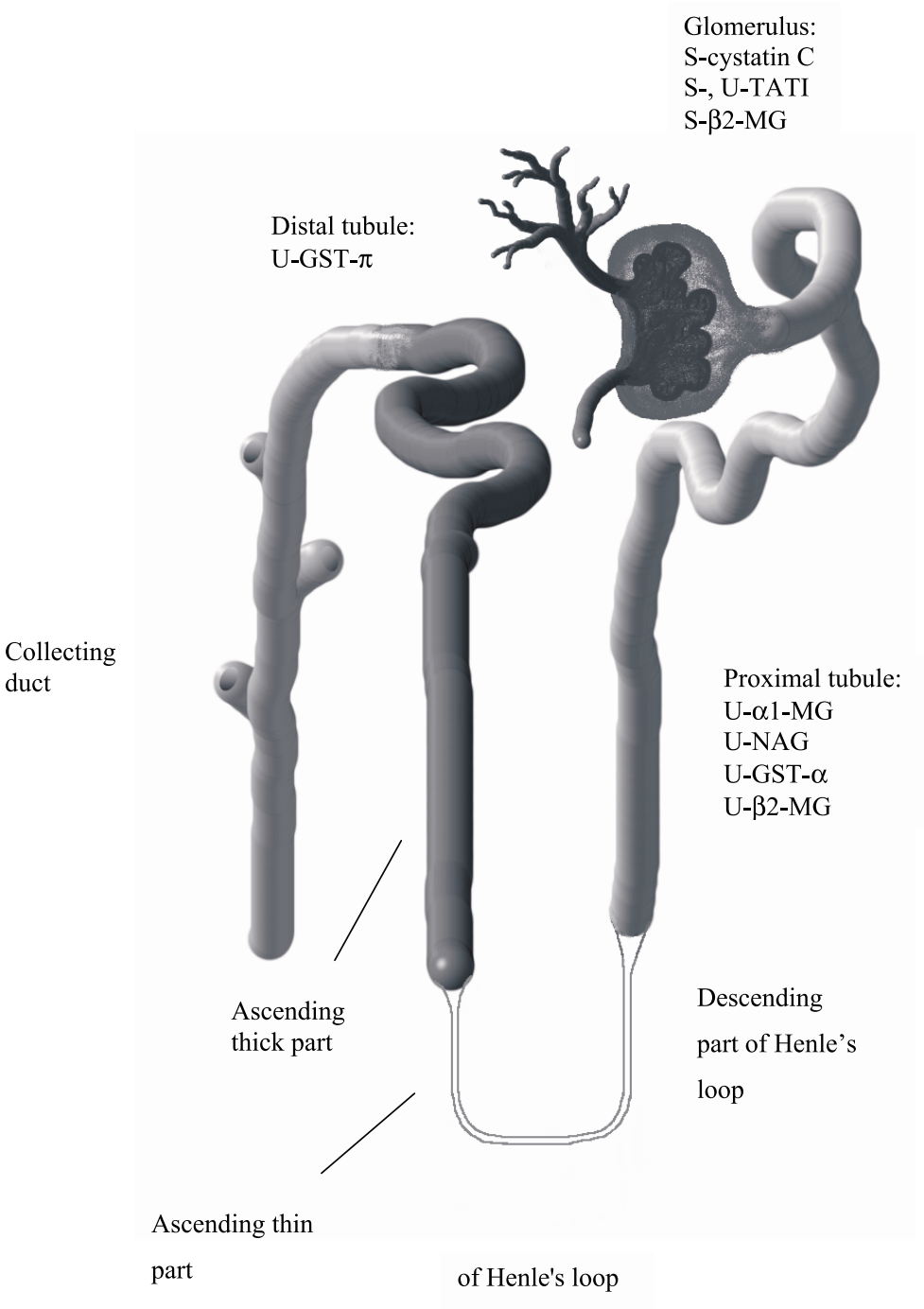
Serum cystatin C increases when GFR decreases. Serum cystatin C can be used in clinical evaluation of GFR in different patient populations because it is not affected by body weight, diet, medications and diseases such as hepatic failure, inflammation and cancer (Grubb et al. 1985, Demirtas et al. 2001). However, glucorticoid treatment is a nonglomerular factor increasing S-cystatin C, and S-cystatin C reference values in the elderly population exceed those reported from adults (Wasén 2004).

α1-MICROGLOBULIN

α1-microglobulin, also called protein HC, is a low molecular weight plasma glycoprotein (M: 26 000–33 000) (Weber and Verwiebe 1992). α1-microglobulin belongs to the lipocalin protein superfamily, and it is synthesized in the liver. In the circulation, approximately half of α1-MG is combined with immunoglobulin A, 7% is bound to albumin and the remaining is unbound (Åkerström et al. 2000). α1-microglobulin passes relatively freely through the glomerular membrane and is reabsorbed by the proximal tubular cells which then catabolize it (Åkerström et al 2000).

Figure 1. Cortical nephron. Presented are the sites of biomarkers release in renal glomerular dysfunction and cellular deterioration.

NAG = N-acetyl-β-D-glucosaminidase, S = Serum, TATI = Tumor associated trypsin inhibitor, U = Urine, α1-MG = α1-microglobulin, GST-α = Glutathione transferase-α, GST-π = Glutathione transferase-π, β2-MG = β2-microglobulin, cystatin C.



Urine α1-MG has been used to measure proximal tubular dysfunction (figure 1). In patients with rheumatoid arthritis, half of the patients had asymptomatic proteinuria and tubular dysfunction, as seen by increased U-α1-MG levels (Niederstadt et al. 1999). In children U- α1-MG excretion increases during urinary infection with pyrexia (Mantur et al. 2000).

During surgery, U-α1-MG has been used to detect early stages of tubular damage and to evaluate renal outcome. Decreased U-α1-MG levels following renal transplantation indicates improved tubular function and rules out developing rejection (Teppo et al. 2000). During cardiac surgery elevated U-α1-MG indicates proximal tubular dysfunction (Gormley et al. 2000).

N-ACETYL-β-D-GLUCOSAMINIDASE

N-acetyl-β-D-glucosaminidase, a lysosomal enzyme (M: 125 000–150 000), is released from the proximal tubular cells in tubular injury (figure 1) (Holdt-Lehmann et al. 2000). Urine NAG can be measured from spot samples or from a 24-hour urine collection (Wellwood et al. 1975, Higuchi et al. 1995). When spot samples are taken, U-NAG is indexed to U-crea to avoid the effect of different urine volumes (Wellwood et al. 1975).

Urine NAG/crea is used in evaluating proximal tubular damage in different clinical settings. Elevated U-NAG/crea-index has been measured after cardiopulmonary bypass and kidney transplantation (Bornstein et al. 1996, Gormley et al. 2000). Exposure to lead has been shown to increase U-NAG levels (Pergande et al. 1994).

β2-MICROGLOBULIN

β2-microglobulin (β2-MG) (M: 12 400), originates from the histocompatibility antigen complex (Grubb et al. 1985). β2-microglobulin is produced by most nucleated cells, especially lymphocytes (Grubb et al. 1985). β2-microglobulin is filtered through the glomerulus and reabsorbed in the proximal tubules. Serum and urine β2-MG increase as tubular damage increases. Serum β2-MG reflects also GFR in early glomerular dysfunction (figure 1) (Schardijn and Statius van Eps 1987).

Serum β2-MG is used to assess renal effects of environmental and work-related cadmium and lead exposure (Jakubowski et al. 2002).

There are some confounding factors to be taken in account when β2-MG is measured. Immunological insults, including stress reactions and infections influence S- β2-MG due to the predominant production of β2-MG in lymphocytes. Also, drugs affecting lymphocytes and their function, e.g. steroid therapy, may alter the β2-MG production (Honkanen et al. 1995).

Diurnal variation of β2-MG production should be taken to account. In healthy patients, maximum S-β2-MG is measured in the morning and in patients with non-treated multiple myeloma, it has been observed that a peak in S-β2-MG occurs in the afternoon (Pasqualetti et al. 1991). Acidic urine, pH less than 6, causes degradation of β2-MG at body temperature and affects U-β2-MG (Schardijn and Statius van Eps 1987).

GLUTATHIONE–S–TRANSFERASE – α AND – π

Glutathione-S-transferases (M: 22 000–29 000) conjugate various molecules, which are detoxified and excreted into the urine (Harrison et al. 1989, Hayes et al. 1991). Glutathione-S-transferases are cytosolic enzymes present in the liver, small intestine, testis, ovaries, adrenal glands and kidneys (Sarvary et al. 2000).

Glutathione-S-transferase -α and -π are considered useful tools in evaluating the site of cellular damage in the kidneys (figure 1) (Usuda et al. 1999, Branten et al. 2000). Glutathione–S–transferase-α is primarily located in the proximal tubules and GST-π is predominantly located in the distal tubules, collecting ducts and loops of Henle. Hence, U-GST-α is a sensitive marker of proximal tubular and U-GST-π of distal tubular damage, respectively (Branten et al. 2000). Both biomarkers have been used to assess renal effects of F^- exposure (Usuda et al. 1999).

TUMOR-ASSOCIATED TRYPSIN INHIBITOR

Tumor-associated trypsin inhibitor (M: 6 000), is expressed in the gastrointestinal, urogenital, and biliary tracts, in the kidney, lung, liver and breast (Stenman 2002). Tumor-associated trypsin inhibitor has the same structure as pancreatic trypsin inhibitor. Tumor-associated trypsin inhibitor is filtered through the glomerulus and metabolized in the proximal tubule (Stenman 2002). When GFR declines, S-TATI increases (Tramonti et al. 1997).

A small amount of TATI is excreted in the urine of healthy humans, but significantly increased amounts of U-TATI are measured in patients with glomerular and tubular dysfunction (Tramonti et al. 1997).

In patients with ovarian tumors, increased S-TATI has been measured (Medl et al. 1995) and S-TATI may also be increased in pelvic inflammatory diseases (Paavonen et al. 1989). These confounding factors should be taken in account when S-TATI is used to measure GFR.

PLASMA RENIN ACTIVITY

Renin is the promoter in the activation of the RAAS. Renin catalyzes the formation of angiotensin I from angiotensinogen. Angiotensin I is then transformed to angiotensin II, which causes vasoconstriction in renal and systemic arterioles (Ganong 2003). Angiotensin II produces relative cortical ischemia directing the blood flow to the medulla (Hall 1986). Activation of angiotensin II releases catecholamines, which cause further vasoconstriction (Giacchetti et al. 1996).

Impaired RBF, diminished renal perfusion pressure and decreased GFR elevates plasma renin activity (P-RA) (Leenen and Stricker 1974). During laparoscopy, pneumoperitoneum increases P-RA (Koivusalo 1997).

Plasma renin activity is a feasible method for measuring the activation of RAAS (Ganong 2003). Plasma renin activity is measured by the amount of angiotensin I produced during blood sample incubation.

SEVOFLURANE

Sevoflurane (M=200) is a liquid volatile anesthetic agent synthesized in 1968. Sevoflurane is fluorinated methyl isopropyl ether (Figure 2) with a boiling point of 58.5° C and a blood-gas partition coefficient of 0.60–0.69 (Wallin et al. 1975). In adults, the end tidal minimum alveolar concentration (MAC) in oxygen in air is 1.7–2.1% and in oxygen in 60% of nitrous oxide 0.7% (Katoh et al. 1987).

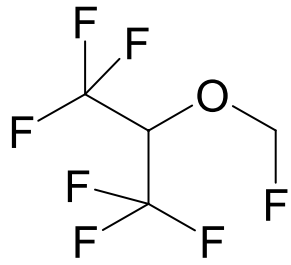


Figure 2. Sevoflurane.

THE METABOLISM OF SEVOFLURANE

Sevoflurane is mainly removed by the lungs and the metabolic rate is moderate (Kharasch et al. 1995a). Less than 5% of sevoflurane is metabolized in the liver by the CYP2E1 to F^- and hexafluoroisopropanol (Kharasch et al. 1995a). Intrarenal defluorination of sevoflurane is minimal (Kharasch et al. 1995b). The metabolism of hexafluoroisopropanol does not release F^- and hexafluoroisopropanol is secreted into the urine as glucuronide conjugates.

The activity of CYP 450 isoenzymes varies between individuals (Piao et al. 2003). In patients with low CYP 2E1 activity, the increase in S-F^- is usually moderate following sevoflurane while in patients with high CYP 2E1 activity, S-F^- may increase significantly after sevoflurane anesthesia (Wandel et al. 1997). This is a risk because high S-F^-, ≥ 50 μmol L^{-1}, is considered to be associated with an increased risk for renal toxicity (Cousins and Mazze 1973).

COMPOUND A

Sevoflurane interacts with dry CO_2-absorbents to produce a series of degradation products: polyvinyl ethers, compound A, B, C, D and E (Bito and Ikeda 1994, Anders 2005). Considering the renal effects, compound A is the most important of the degradation products. The production of compound A is temperature-dependent. The temperature in CO_2-absorbents increases when low fresh gas flow is used or when CO_2 production is increased (Fang et al. 1995).

In experimental studies, metabolites of compound A have caused renal proximal tubular injury, but these results cannot be directly applied to humans. In rats, compound A is metabolized by renal cysteine β-lyases to nephrotoxic metabolites (Kharasch et al. 1999). In human kidneys, the β-lyase pathway is less active than in rats (Kharasch et al. 1999), and thus, the risk of renal toxicity should be low in humans.

Frink and colleagues (1992) measured degradation products after three hours low flow (fresh gas flow of 1 L min^{-1}) sevoflurane anesthesia with sodalime or baralyme as CO_2-absorbent in humans. Compound A was the only detectable degradation product and the mean concentrations with sodalime and with baralyme were 8 and 20 parts per million, respectively (Frink et al. 1992). In Finland, sodalime absorbents are mainly used.

SEVOFLURANE ANESTHESIA AND RENAL MARKERS

N-acetyl-β-D-glucosaminidase

The renal glomerular and tubular effects of prolonged inhalation anesthesia are described in several studies, although the results are contradictory (table 1). Higuchi and co-workers (1995) compared the proximal tubular effects of 9–14 MAC-hour sevoflurane and isoflurane anesthesia in patients undergoing orthopedic surgery. Urine NAG/crea was significantly higher in those patients with sevoflurane anesthesia who had $S\text{-}F^-$ 50 μmol L^{-1} or higher when compared with patients anesthetized with isoflurane with $S\text{-}F^-$ of 5 μmol L^{-1}. However, no changes in clinical renal function were noted (Higuchi et al. 1995).

In other studies opposite results have been presented. After prolonged (16–18 MAC-hour) anesthesia, there were no differences in U-NAG excretion in patients anesthetized with either sevoflurane or isoflurane. Urine NAG/crea increased from a mean of 5 units g^{-1} at baseline with both agents to the highest concentration of 30 units g^{-1} measured at postoperative day 5 (Obata et al. 2000). In two other studies, no increases of U-NAG were noted after 3.6 and 10 MAC-hour sevoflurane anesthesia (Kharasch et al. 1997, Ebert et al. 1998a). In patients with moderately impaired renal function, crea clearance 0.17–0.92 mL s^{-1}, U-NAG did not increase from the baseline after 5 MAC-hour sevoflurane or isoflurane anesthesia (Tsukamoto et al. 1996).

β2-microglobulin

Urine β2-MG has been used to evaluate the proximal tubular effect of inhalation anesthesia. Increased U-β2-MG has been measured after 11 MAC-hour sevoflurane anesthesia (Higuchi et al. 1998). In a small study, U-β2-MG increased compared to the baseline after sevoflurane inhalation, but repeated sevoflurane anesthesia did not affect U-β2-MG (Nishiyama et al. 1998).

Patients with moderately impaired renal function, crea clearance 0.17–0.92 mL s^{-1}, and chronic renal failure receiving hemodialysis were anesthetized with 5 MAC-hour sevoflurane without any significant changes in U-β2-MG (Nishiyama et al. 1996, Tsukamoto et al. 1996).

Glutathione transferase-α and -π

Urine GST-α and- π have been used to detect possible renal tubular damage after sevoflurane anesthesia with controversial results. In one study, 10 MAC-hour sevoflurane anesthesia resulted in increased U-GST-α and- π with albuminuria and glucosuria for one to three days after anesthesia (Eger et al. 1997). These findings have not been confirmed by others. After 10 MAC-hour sevoflurane anesthesia, using a similar study setting, the U-GST-α and -π excretion was slightly elevated at 48 hours after anesthesia but both biomarkers returned to normal at 72 hours without significant glucosuria or albuminuria (Ebert et al. 1998a). In another trial, there were no significant differences in U-GST-α/crea and -π/crea in patients anesthetized with 3–4 MAC-hour isoflurane or sevoflurane anesthesia (Kharasch et al. 1997).

Table 2. Effects of prolonged inhalation anesthesia (≥ 6 MAC-hour) on renal function

Author	Number of patients	Inhalation anesthetic	Fresh gas low (L min^{-1})	MAC-hour	S-F$^-$ (μmol L^{-1})	Renal markers	
						Sevoflurane	Other inhalation anesthetic
Bito et al. (1997)	48	Isoflurane, Sevoflurane	Sevo: 1 Iso or Sevo: 6-10	7	not reported	U-NAG/crea ↑ at 72 h	Isoflurane: U-NAG/crea ↑ at 72 h after anesthesia
Ebert et al. (1998a)	8	Sevoflurane	1	6	mean 50 (SD 9)	U-GST-π ↔	
Ebert et al. (1998b)	13	Sevoflurane	2	10	mean 66 (SD 15)	U-NAG ↔, U-GST-α ↑ at 24 and 48 h, U-GST-π ↑ at 24 h	
Eger et al. (1997)	7	Sevoflurane, Desflurane	2	10	maximum 125, mean 71 (SD 13)	U-GST-α ↑ at 48 and 72 h U-GST-π ↑ at 72 h	Desflurane: U-GST-π ↑ at 48 h
Frink et al. (1994)	14	Sevoflurane, Enflurane	not reported	10	Sevo: mean 47 (SD 3) Enflurane: mean 23 (SD 1)	U-NAG/crea ↔	Enflurane: U-NAG/crea ↔ renal concentrating function↓
Higuchi et al. (1995)	34	Sevoflurane, Isoflurane	6	9–14	mean 58 (SD 4)	if S-F$^-$ ≥ 50 μmol L^{-1} U-NAG/crea↑ at 48 and 72 h	Isoflurane: U-NAG/crea ↔
Higuchi and Adachi (2002)	37	Sevoflurane	1	7	not reported	U-NAG/crea ↔, U-β2-MG ↔	
Higuchi et al. (1998)	42	Sevoflurane, Isoflurane	Sevo: 1 Iso or Sevo: 6	9	not reported	U-NAG/crea ↑ at 24 to 120 h	Isoflurane: U-NAG/crea ↔
Kharasch et al. (2001)	55	Sevoflurane, Isoflurane	<1	9	not reported	crea clearance ↔	Isoflurane: crea clearance ↔
Mazze et al. (1977)	19	Enflurane, Halothane	not reported	10–14	mean 34 (SD 3)		Enflurane: S-crea ↑ crea clearance ↓ renal concentrating function ↓ Halothane: ↔
Obata et al. (2000)	30	Sevoflurane, Isoflurane	Sevo: 1 Iso or Sevo: 6–10	16–18	mean 54 (SD 5) μmol L^{-1}	U-NAG/crea ↑ at 24 to 120 h after anesthesia	Isoflurane: U-NAG/crea ↑ at 24 to 120 h after anesthesia

MAC-hour = minimum alveolar concentration-hour, des = desflurane, iso = isoflurane, sevo = sevoflurane, SD = standard deviation, U = urine, S = serum, crea = creatinine, NAG = N-acetyl-β-D glucosaminidase, GST-α = glutathione transferase -α, GST-π = glutathione transferase-π, β2-MG = β2-microglobulin, ↑ = increase, ↓ = decrease, ↔ = remained at baseline.

ENFLURANE

Enflurane (M=185) is a volatile liquid anesthetic (Figure 3) with a boiling point of 56.5° C and a blood-gas partition coefficient of 1.9. The enflurane's MAC in oxygen in air is 1.7% (end tidal concentration) and in oxygen in 70% nitrous oxide 0.6% (Gion and Saidman 1971).

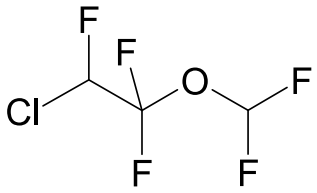


Figure 3. Enflurane.

METABOLISM OF ENFLURANE

2% of enflurane is metabolized, predominantly in the liver by CYP2E1, to F^- (Kharasch et al. 1994). In healthy volunteers, the renal concentrating capacity decreases with S-F^- of 34 μmol L^{-1} after enflurane anesthesia (Mazze et al. 1977). Reactive intermediate metabolic products may also be potentially nephrotoxic. Alkaline degradation of enflurane produces halogenated alkenes that are conjugated further to possibly nephrotoxic thiol compounds (Orhan et al. 2001). Fluoride is excreted in the urine and high U-pH increases F^- clearance (Oikkonen 1981), decreasing possible renal toxicity.

INORGANIC FLUORIDE FORMED IN THE METABOLISM OF ENFLURANE AND SEVOFLURANE AND RENAL FUNCTION

Fluorine substitution can alter the chemical properties, metabolism, disposition (distribution of the drug, drug clearance and routes of clearance) and biological activity of drugs (Park et al. 2001). Fluorine forms a strong bond with carbon in flurane-type inhalation anesthetics. Fluorine may increase lipophilicity of an inhalation anesthetic and passive diffusion across membranes and facilitating penetration to the central nervous system. Fluoride substitution may alter the metabolism and toxicity of the drug. This can be noted in the development of modern inhalation anesthetics. Extensive release of F^- in the metabolism of methoxyflurane caused high urine output syndrome and even fatal renal failure. By increasing fluorine substitution, as in enflurane and isoflurane, a reduction of metabolism and defluorination can be achieved (Park et al. 2001). The metabolism of sevoflurane produces relatively high S-F^-, but severe renal failure has not been reported (Mazze et al. 2000).

Inorganic fluoride induces renal failure by inhibiting enzymes responsible of glycolysis and energy production. The adenosine triphosphatase enzyme and membrane transport is impaired (Oikkonen 1984). Fluoride toxicity causes ADH resistant polyuria, so it may be assumed that the F^- dissipates the concentration gradient in the renal medulla inhibiting sodium and chloride reabsorption into the ascending loop of Henle. Also, the ADH mediated water reabsorption in the collecting duct is inhibited (Rush and Willis 1982). Fluoride induces inhibition of salt and water reabsorption in the proximal tubule (Mazze 1976). In acidic milieu, hydrogen F (weak acid pKa 3.3) is mostly nonionized and is easily absorbed into tubular cells to be filtered again in the glomerulus, which increases the potential for renal toxicity. If urine pH is increased, a greater fraction of F is ionized and is excreted into the urine, hence, becoming harmless (Järnberg et al 1981).

NONSTEROIDAL ANTI-INFLAMMATORY ANALGESIC DRUGS

The mechanism of action of NSAIDs is based on their ability to reduce the formation of PGs (Vane 1971). This ability is associated with inhibition of the cyclo-oxygenase (COX) enzyme, which converts arachidonic acid to different prostanoids. The prostanoid family includes PGD_2, PGE_2, $PGF_{2\alpha}$, prostacyclin (called also PGI_2), and thromboxane A_2. The COX enzymes exist in at least two isoforms, COX-1 and COX-2 (Vane 1994). Cyclo-oxygenase-1 is constitutively expressed and responsible of production of physiologically important prostanoids. Cyclo-oxygenase -2 is rapidly up-regulated by inflammatory stimuli. Cyclo-oxygenase-2 is also constitutively expressed in the central nervous system, reproductive organs, and kidneys, and has physiological roles such as maintaining the fluid balance in the kidneys (Kammerl et al. 2001). Recently, a variant of COX-1 has been detected in the brain and named COX-3. Some of the therapeutic actions of paracetamol are supposed to be mediated by COX –3 (Chandrasekharan et al. 2002).

Conventional NSAIDs prevent the function of both COX-1 and -2 isoforms and the COX-1 sparing NSAIDs (coxibs) inhibit COX-2 at lower concentrations than COX-1. NSAIDs are widely used to treat mild postoperative pain after minor surgery (Nikanne et al. 1999), and in adjunct to opioids and other pain relieving modalities for moderate to severe pain after major surgery (Kokki et al. 1999). Many NSAIDs are also available in a parenteral form which may facilitate perioperative administration.

NSAIDS AND RENAL FUNCTION

In non-surgical hospitalized patients with NSAIDs, the incidence of ARF varies from 1 to 4% (Feldman et al. 1997, Whelton 2000). In high-risk patients with PG dependent renal function (e.g. patients with hypovolemia, heart failure, liver cirrhosis or underlying renal disease), NSAIDs reduce RBF and GFR in a dose-dependent fashion and ARF may ensue (Delmas 1995). Also, patients using angiotensin converting enzyme inhibitors or angiotensin receptor agonists in combination with NSAIDs are at risk of renal deterioration. Many mechanisms are included,

with inhibition of PG dependent vasodilatation of the afferent arteriole and angiotensin-mediated vasoconstriction of the efferent arteriole being the most important (Juhlin et al. 2004, Loboz and Shenfield 2005).

Kidneys produce PGE_2 and PGI_2. In normal situations, PGs may have only a minor role in maintaining renal function, but in patients with hypovolemia, catecholamines induce production of renal PGs, which vasodilate the afferent renal vessels, thus preserving RBF and function (Yared et al. 1985). If the PG synthesis is prevented by NSAIDs in hypovolemic patients, angiotensin II, ADH and catecholamines may cause vasoconstriction in renal vessels, and RBF and GFR are declined. If vasoconstriction of afferent vessels is maintained, renal failure may eventually ensue.

In patients with perioperative NSAIDs, minor changes in renal function are common and some degree of fluid and sodium retention occurs in nearly all patients (Lee et al. 2004). More serious complications may also occur with long-term NSAID use. Hyperkalemia, reversible nephrotic syndrome with interstitial nephritis, permanent papillary necrosis and interstitial scarring are reported in patients on a long-term NSAID regimen (Whelton and Hamilton 1991, Rocha et al. 2001). Other structural changes caused by NSAIDs are tubular necrosis and renal interstitial infiltration of lymphocytes (Kleinknecht et al. 1986).

The incidence of NSAID-related renal structural alterations appears to be low, although the true number of patients may be significant considering the wide use of NSAIDs. According the Royal College of Anaesthetists guidelines (1998), renal function should be monitored regularly in at risk patients undergoing major surgery when NSAIDs are administered during the perioperative period.

KETOROLAC

Ketorolac (M=376), a pyrroleacetic acid derivative (Figure 4), is a nonselective NSAID that has been marketed in Finland since 1991. Ketorolac is available as a parenteral form, which enables the perioperative i.v. (intravenous) and i.m. (intramuscular) use. When injected intramuscularly, the peak concentration of ketorolac occurs 50 minutes after injection, and the elimination half-life in healthy persons is 5 hours. The analgesic effect of ketorolac is noted 30 minutes post-injection (Maunuksela et al. 1992). Ketorolac is eliminated by glucuronidisation in the liver and excreted by the kidneys. The metabolites have no analgesic effect (Gillis and Brogden 1997).

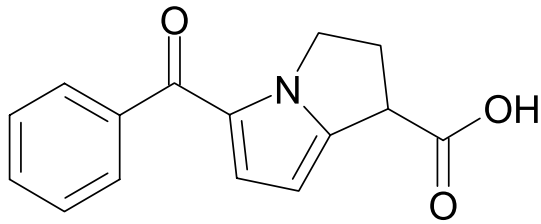


Figure 4. Ketorolac.

Ketorolac is used to treat moderate and severe postoperative pain. Ketorolac is effective in treating pain after laparoscopic surgery (Lane et al. 1996) and laparotomy (Barton et al. 2002). After hip replacement, ketorolac was as effective as diclofenac and ketoprofen (Kostamovaara et al. 1998).

Ketorolac may cause adverse reactions typical for NSAIDs. After major surgery, perioperative bleeding from the surgical site and gastrointestinal tract, allergic reactions and ARF are reported with the same incidence in patients receiving ketorolac, ketoprofen or diclofenac (Forrest et al. 2002). After major surgery, renal impairment is detected in 0.1% of patients receiving ketorolac, ketoprofen or diclofenac (Forrest et al. 2002). According to the marketing authorization holder, the maximum recommended daily dose of ketorolac is 120 mg, and the administration should not exceed two days. The use of ketorolac for more than five days may be associated with increased incidence of ARF (Feldman et al. 1997).

CLONIDINE

Clonidine (M=267), a 2-imidazoline derivative, is an adrenoreceptor agonist (Figure 5). Clonidine has a greater affinity to presynaptic α2-adrenergic receptors than postsynaptic α1-receptors, the α2/α1-selectivity ratio of 220 (Virtanen et al. 1988).

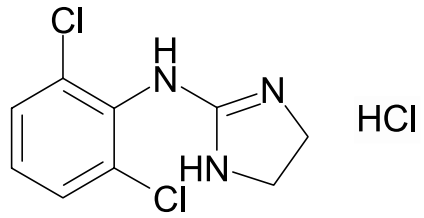


Figure 5. Clonidine hydrochloride.

The bioavailability of oral clonidine is 75% and the elimination half-life of clonidine varies between 10–20 hours. Half of clonidine is eliminated unchanged, mainly by the kidneys, and 20% of clonidine is secreted into the feces (Lowenthal 1980).

Clonidine acts centrally, at the medulla oblongata, and peripherally. Clonidine reduces sympathetic tone. Consequently, peripheral vascular resistance decreases resulting in a fall in diastolic and systolic blood pressure and a reduction in heart rate (Kallio et al. 1990). The dose response of clonidine on blood pressure follows a U-shaped curve. Low doses of clonidine (2 μg kg^{-1} or less) do not affect blood pressure and high doses (6 μg kg^{-1} or more) with peripheral vasoconstriction from high circulating drug concentrations oppose central sympatholysis (Eisenach et al. 1996).

Clonidine decreases cardiac output. This effect is based on decreased heart rate, as stroke volume is not affected (Kallio et al. 1990). However, during CO_2 pneumoperitoneum high dose of clonidine, 8 μg kg^{-1}, does not affect cardiac output in normovolemic patients (Joris et al. 1998).

CLONIDINE AND RENAL FUNCTION

In the kidney, clonidine inhibits fluid reabsorption in the proximal tubules (Rouse et al. 1990) and subsequently increases sodium and water excretion (Gellai and Ruffolo 1987). Kulka and co-workers (1996) demonstrated that clonidine maintains renal function after cardiac surgery. Although clonidine decreases cardiac output, it does not decrease GFR and RBF (Barendregt et al. 1994).

The renal effect of clonidine may be based on decreased vasoactive hormones that affect renal vasoconstriction. The effect of ADH on renal collecting tubules is inhibited by clonidine (Gellai and Edwards 1988). Clonidine also inhibits the central ADH secretion (Peskind et al. 1987), but conflicting results exist (Pouttu et al. 1987). During isoflurane anesthesia, ADH secretion was similar but mean urine output was doubled in the patients receiving clonidine (5 μg kg^{-1}), as compared with the control patients (Hamaya et al. 1994).

Clonidine (8 μg kg^{-1}) reduces the release of noradrenaline during laparoscopy (Joris et al. 1998). During major vascular surgery, serum concentrations of adrenaline, noradrenaline and ADH are low in patients receiving clonidine (7 μg kg^{-1}) (Quintin et al. 1991).

CIRCULATORY EFFECTS OF CARBON DIOXIDE PNEUMOPERITONEUM

Due to its physical safety, CO_2 is used for creation of pneumoperitoneum in laparoscopic surgery. Carbon dioxide is a normal product of human metabolism and it is non-toxic in physiological concentrations (Lenfant and Aucut 1965). However, CO_2 has direct and indirect hemodynamic effects. Carbon dioxide dilates peripheral arterioles and depresses myocardial contractility. Indirectly CO_2 activates the central nervous system and evokes sympathoadrenal responses causing tachycardia (Cullen and Eger 1974). Carbon dioxide pneumoperitoneum increases arterial blood pressure and peripheral vascular resistance and reduces cardiac output (Hirvonen et al. 1997). In patients without fluid preloading, stroke volume and cardiac output decrease after head up tilt and CO_2-insufflation. These changes resolve after laparoscopy (Joris et al. 1998). On the contrary, in healthy patients (Andersson et al. 2003) or patients with appropriate hydration therapy prior to the pneumoperitoneum, cardiac output increases after CO_2-insufflation (Bäcklund et al. 1998).

CARBON DIOXIDE PNEUMOPERITONEUM AND RENAL FUNCTION

In normal conditions the abdominal cavity is pressureless. In experimental studies where renal vein pressure was increased by mechanical compression of renal vessels, RBF and GFR decreased (McDougall et al. 1996, Doty et al. 2000). When the abdominal cavity is pressurized to an intra-abdominal pressure (IAP) of 15 mmHg, which is higher than the normally suggested maximum of 12 mmHg, the renal vascular resistance increases and RBF and GFR decrease (Chiu et al. 1995). During laparoscopy, the harmful effects of the compression of renal vessels by pneumoperitoneum on renal function are enhanced by the simultaneous neuroendo-

crinological changes induced by surgical stress such as increased P-RA and catecholamine release (Koivusalo 1997).

Urine output decreases during laparoscopic surgery, but recovers after pneumoperitoneum (Chiu et al. 1995, Koivusalo 1997). The reason of impaired urine output is multifactorial and not fully understood (Koivusalo 1997). The release of vasoactive substances during pneumoperitoneum may contribute also to decreased urine output. Adrenaline, noradrenaline and ADH are increased during pneumoperitoneum (Viinamäki and Punnonen 1982, Koivusalo 1997, Joris et al. 1998). Vasoconstriction induced by the catecholamines and the effect of ADH are other reasons for decreased urine output during laparoscopy.

SMOKING AND FLUORIDE RELEASE FROM ENFLURANE AND SEVOFLURANE

Smoking is common in Finland and it is reported that 15–20% of Finnish women smoke cigarettes regularly (Uutela and Koskinen 2002). Tobacco smoke consists of numerous components, some of which have been investigated regarding their pharmacological effects on the body (Miller 1990). Tobacco smoke may also interact with drugs and potentially alter their pharmacokinetic and pharmacodynamic properties (Zevin and Benowitz 1999).

Tobacco smoke may both inhibit and induce drug metabolism. Nicotine and its main metabolite cotine inhibit CYP 2EI in the liver (Van Vleet 2001). CYP 2E1 is the isoenzyme responsible for defluorination of fluorinated ether anesthetics (Kharasch and Thummel 1993, Kharasch et al. 1994) and increased CYP 2E1 activity may increase inhalation anesthetic metabolism and result in a quicker and smoother recovery from anesthesia (Sweeney 2004). The CYP 2E1 enzyme metabolizes ethanol and paracetamol although smoking does not inhibit their metabolism (Zevin and Benowitz 1999).

On the other hand, nicotine induces several microsomal enzymes such as CYP 2A1/2A2 and CYP 2B1/2B2 (Zevin and Benowitz 1999). Induction of liver enzymes in smokers increases the metabolism of numerous drugs (Vähäkangas et al. 1983, Miller 1990). Tobacco smoke consists of polycyclic aromatic hydrocarbons, which are formed as a result of incomplete combustion of organic materials (Miller 1990). Polycyclic hydrocarbons, e.g. anthracine and benzopyrene, are also potent inducers of drug metabolism (Jusko 1979).

AIMS OF THE STUDY

The aim of the present study was to assess renal function and cellular integrity during and after anesthesia and surgery using sensitive biomarkers of glomerular function, tubular function and cellular damage. Special emphasis was placed on determining whether F^- derived from the metabolism of enflurane and sevoflurane, as well as whether concomitant use of ketorolac or clonidine with inhalation anesthesia would result in changes in renal function or cellular damage. Sensitive biomarkers were used in order to also detect minor deteriorations.

The specific aims of the present study were:

1. To assess the effects of perioperative ketorolac on renal function and cellular integrity (Study 1).

2. To assess the effects of F^- on renal function and cellular integrity after enflurane and sevoflurane anesthesia (Study 3, 4).

3. To assess the effects of cigarette smoking on production of F^- in patients with enflurane and sevoflurane anesthesia (Study 3, 4).

4. To assess the effects of preoperative clonidine on renal function and cellular integrity during laparoscopic surgery (Study 2).

PATIENTS AND METHODS

The study was carried out in Surgical Hospital and Women's Hospital in Helsinki University Hospital and in Kuopio University Hospital. A total of 143 patients with ASA (American Society of Anesthesiologists) physical status I–II (American Society of Anesthesiologists 2006) undergoing conventional or endoscopic surgery were included. Written informed consent was obtained from each patient. The study protocols were accepted by the ethics committees of the hospitals. The studies were conducted according to good clinical practice guidelines (European Agency for the Evaluation of Medicinal Products 2005) and Declaration of Helsinki (World Medical Association 2004). In studies 1–2, patients were randomly allocated to one of two study groups. In studies 3–4, two parallel cohorts of women, non-smokers and smokers, were compared (table 3). None of the enrolled patients was excluded.

Table 3. The characteristics of the studies 1–4.

	Patients	Design	Type of surgery	Main purpose
Study 1, publication I and II	30 women	Prospective, randomized, double-blinded, placebo-controlled	Mammary resection/ablation and evacuation of axillae	Renal effects of ketorolac during sevoflurane anesthesia
Study 2, publication III	21 women 9 men	Prospective, randomized, double-blinded, placebo-controlled	Laparoscopic cholecystectomy	Renal effects of clonidine during pneumoperitoneum for laparoscopic surgery
Study 3, publication IV	33 women	Prospective, open, with parallel groups	Gynecological operations; abdominal, laparoscopic, vaginal	Effects of tobacco smoking on enflurane metabolism and renal function
Study 4, publication V	50 women	Prospective, open, with parallel groups	Gynecological operations; abdominal, laparoscopic, vaginal	Effects of tobacco smoking on sevoflurane metabolism and renal function

STUDY DESIGN

The exclusion criteria were allergy or other contraindications for drugs used, obesity (body mass index over 32 kg m^{-2}), pre-existing renal and/or hepatic diseases, recent use of NSAIDs (study 1), use of angiotensin converting enzyme inhibitors and angiotensin receptor antagonists (study 2), and concomitant medication known to affect CYP450 activity (study 4–5).

The blood and urine biomarkers of renal function and integrity as well as the collection times for each of the four studies are listed in table 4.

Study 1, publication I: Thirty women, aged 28–60 years, were given three i.m. doses of either ketorolac 30 mg or normal saline as placebo 60 minutes prior to, at the end of and six hours after sevoflurane anesthesia (table 3).

Blood and urine samples for the analyses of S- and U-F^- and U-crea were collected at baseline, at 2 hours of the anesthesia, and at 2, 12, 24 and 48 hours after the end of sevoflurane inhalation. Blood hemoglobin and hematocrit were measured at baseline and at 24 hours after the anesthesia. Urine samples for the analyses of renal biomarkers were collected at timepoints shown in the table 4. Urine α1-MG is a marker of proximal tubular function and U-NAG of proximal tubular cellular deterioration.

Study 1, publication II: In the second publication of the study 1 additional renal glomerular and tubular biomarker concentrations were reported (table 4). Urine GST-α and -π are biomarkers of proximal and distal tubular cellular deterioration, respectively.

For 10 patients from each group S-cystatin C, a biomarker of GFR, was measured at baseline and at 48 hours after the anesthesia (table 4).

Study 2, publication III: Thirty patients, 21 women and 9 men aged 27–59 years, were given clonidine 4.5 μg kg^{-1} or normal saline as a placebo i.m. 60 minutes before anesthesia (table 3).

Blood and urine samples for assessment of U-crea were collected at baseline, at 15, 30, 60 minutes after the induction of pneumoperitoneum, and at 1, 3, and 24 hours after the pneumoperitoneum. Urine NAG, biomarker of proximal tubular deterioration, S-ADH and P-RA were sampled at timepoints shown in the table 4.

Study 3, publication IV: Sixteen non-smoking and 17 smoking (over 10 cigarettes per day) patients, aged 27–59 years, (table 3) were given enflurane at 1.3 MAC (end tidal concentration of enflurane 2.1% in 33% oxygen in air) for 45 minutes, which corresponds to one MAC-hour enflurane anesthesia. After 45 minutes, anesthesia was maintained with propofol infusion.
Blood samples for S-F^- were collected at baseline and at 1, 2, 3, 6, 12 and 24 hours after the anesthetic inhalation. Serum for S-crea was collected at baseline, and at 24 and 48 hours after the anesthesia. Serum TATI and β2-MG, biomarkers of glomerular function, and U-TATI and U-β2-MG, biomarkers of proximal tubular deterioration, were sampled at timepoints shown in table 4.

Study 4, publication V: The study protocol was similar to study 3, but instead of enflurane, the 25 non-smoking and 25 smoking (over 10 cigarettes daily) patients, aged 19–68, years were given one MAC-hour sevoflurane anesthesia (table 3).

The patients received sevoflurane at 1.3 MAC (end tidal concentration of sevoflurane 2.7% in 33% oxygen in air) for 45 minutes, which corresponds to one MAC-hour sevoflurane anesthesia.

Table 4. Sampling times of renal biomarkers

		During anesthesia				Recovery period					
	Baseline	**15 min**	**30 min**	**60 min**	**120 min**	**1 h**	**2h**	**3h**	**12 h**	**24h**	**48h**
		of pneumoperitoneum									
Study 1	U-NAG U-α1-MG S-, U-β2-MG U-GST-α U-GST-π S-Cyst C				U-NAG U-α1-MG S-,U-β2-MG U-GST-α U-GST-π		U-NAG U-α1-MG S-,U-β2-MG U-GST-α U-GST-π		U-NAG U-α1-MG S-,U-β2-MG U-GST-α U-GST-π	U-NAG U-α1-MG S-,U-β2-MG U-GST-α U-GST-π	U-NAG U-α1-MG S-,U-β2-MG U-GST-α U-GST-π S-Cyst C
Study 2	U-NAG P-RA S-ADH	U-NAG P-RA S-ADH	U-NAG P-RA S-ADH	U-NAG P-RA S-ADH		U-NAG P-RA S-ADH		U-NAG P-RA S-ADH		U-NAG P-RA S-ADH	
Study 3 and 4	S-,U-β2-MG S-,U-TATI									S-,U-β2-MG S-,U-TATI	S-,U-β2-MG S-,U-TATI

NAG = N-acetyl-β-D-glucosaminidase, α1-MG = α1-microglobulin, β2-MG = β2-microglobulin, GST-α = Glutathione transferase-α, GST-π = Glutathione transferase -π, Cyst C = cystatin C, TATI = Tumor associated trypsin inhibitor, ADH = Antidiuretic hormone, RA = renin activity, P = plasma, S = Serum, U = Urine.

ANESTHESIA MANAGEMENT

Anesthesia was standardized in each study. Diazepam 5–10 mg was given for premedication by mouth 60 minutes before anesthesia. Glycopyrrolate (0.2 mg, study 1, or 0.4 μg kg^{-1}, study 2) and fentanyl or alfentanil was administered i.v. (study 2). Propofol (2–3 mg kg^{-1}) i.v. was used for anesthesia induction. Tracheal intubation was facilitated with cis-atracurium (study 1 and 2) or rocuronium (study 3 and 4). At the end of surgery, neostigmine (2.5 mg) and glycopyrrolate (0.5 mg) was given to reverse muscle relaxation. Anesthesia was maintained with sevoflurane (study 1), isoflurane (study 2), enflurane for 45 minutes followed by propofol infusion (study 3) and sevoflurane 45 minutes followed by propofol infusion (study 4).

Fluid therapy was standardized. In study 1, Ringer's acetate and 6% hydroxyethyl starch infusion were used to maintain central venous pressure at 6–12 mmHg. In study 2, patients were hydrated with Ringer's acetate infusion (8 mL kg^{-1}) i.v. for 20 minutes followed by an additional infusion (10 mL kg^{-1} h^{-1}) and 500 mL of 6% hydroxyethyl starch. In studies 3 and 4, Ringers' acetate infusion was given (10 mL kg^{-1}) at the induction of anesthesia and followed by an infusion (5 mL kg^{-1} h^{-1}) during surgery and recovery room stay. From the first postoperative day all patients were allowed to drink freely.

ANALYTICAL METHODS

The concentrations of F^- were determined by a method modified from that of Fry and Taves (1970). A fluoride selective combination electrode was used for the measurement. The sensitivity of the assay was 0.5 μmol L^{-1} and the interassay coefficient of variation was less than 8%.

The activity of U-NAG was determined by using 3-cresonsulphonphtalein-N-acetyl-β-glucosamine as a substrate (Boehringer Mannheim Biochemica, Mannheim, Germany) (Noto et al. 1983). The activity of NAG was normalized to U-crea and expressed as U-NAG/crea (units of U-NAG activity per gram or mmol of U-crea) (Wellwood et al. 1975).

Serum and urine β2-MG samples were analyzed using time-resolved fluoroimmunoassay with dissociation enhanced lanthanide fluoroimmunoassay β2- microkit® (Delfia® Wallac Oy, Turku, Finland). The analyses of S- and U-sodium, -potassium and -crea as well as S- and U-osmolality and S-urea were performed in the laboratory of clinical chemistry of the Helsinki University Hospital (Hospital District of Helsinki and Uusimaa, Huslab 2005) or Laboratory Centre, Kuopio University Hospital (Kuopio University Hospital, Laboratory Centre 2005).

Serum cystatin C was determined using a DAKO Cystatin C PET kit (Dako Inc., Copenhagen Denmark) at United Laboratories Ltd., Helsinki, Finland. Urine α1-MG was analyzed by radioimmunoassay according the method described by Teppo and co-workers (2000). Urine GST-α was analyzed by enzyme immunoassay using Nephkit™-Alpha, and U-GST-π using Biotrin Nephkit™-Pi (Biotrin International Ltd., Sinsheim-Reihen, Germany). To eliminate the influence of variations in urine volume, α1-MG, GST-α and -π were normalized to U-crea taken from spot samples. Urine crea was measured by the modified Jaffe reaction (Junge et al. 2004, Hospital District of Helsinki and Uusimaa, Huslab 2005), and S-and U-phosphate were measured by a photometric method (Anner and Mossmayer 1975).

The reference value for S-cystatin C < 1.2 mg L^{-1} in adults aged 50 years or less and < 1.4 mg L^{-1} in adults older than 50 years. The reference values for S-crea is 60–100 μmol L^{-1} for men and 50–90 μmol L^{-1} for women and for S-urea, 3.5–8.1 mmol L^{-1} for men and 3.1–7.9 mmol

L^{-1} for women (Hospital district of Helsinki and Uusimaa 2005). The reference values for U-α1-MG/crea are 0.27 (0.04–0.70) g mol^{-1} (mean and minimum-maximum), U-GST-α/crea 0.61 (0.10–1.9) and U-GST-π/crea 2.4 (0.25–7.4) mg mol^{-1}. Reference values for U-α1-MG/crea were derived from 33 healthy individuals (23–63 years) (Teppo et al. 2000) and U-GST-α/crea and -π/crea from 38 adult men age of 18–46 years (Teppo A-M, personal communication 2000). The reference values obtained from 13 healthy men and 45 women for uncorrelated U-NAG is 0.65–6.1 units L^{-1} (Koivusalo 1997).

Plasma RA and S-ADH were analyzed by radioimmunoassay (Fyhrquist et al. 1976).

Serum and U-TATI were analyzed by radioimmunoassay (Orion Diagnostica, Espoo, Finland). The reference values for S-TATI are 0–2 nmol L^{-1} (pathological if > 3 nmol L^{-1}), for U-TATI indexed to U-crea 0–0.64 10^{-6}, and for S-β2-MG 0.6–3.0 mg L^{-1} and for U-β2-MG less than 0.25 mg L^{-1}, respectively (Hospital district of Helsinki and Uusimaa 2005, Kuopio University Hospital, Laboratory Centre 2005).

STATISTICAL ANALYSIS

Parametric tests were used for variables with normal distribution and nonparametric tests for variables with skewed distributions. The Friedman test was used to compare S-F^- and urine marker concentrations observed over the different sample times and Wilcoxon signed ranks test for pair-wise comparisons (study 1–4). For differences between the groups concerning continuous data, the unpaired t-test and Mann-Whitney U-test were used. For categorical variables, the chi squared-test was used. Correlations were calculated using the Pearson correlation coefficient. A p-value of 0.05 or less was considered statistically significant.

In the original publications (I–III) parametric tests were used, but because the distribution of the data was skewed, the statistics for studies 1 and 2 were recalculated using nonparametric tests.

The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 11.5, SPSS Inc., Chicago, USA).

RESULTS

The total number of patients was 143. The patient characteristics and the type of surgery are presented in tables 5 and 6. The anesthesia and fluid therapy were standardized and these protocols were used without any major deviations considered to affect the study parameters.

Table 5. Patient characteristics, data are median (minimum - maximum) or number of cases.

	Age (years)	Smoking no/yes	Cigarettes 10–20/day	ASA I/II	Type of surgery
Study 1 Ketorolac (n=15)	50 (38–60)	12/3	3	12/3	Mastectomy 4 Minor breast surgery 11
Control (n=15)	48 (28–56)	8/7	7	12/3	Mastectomy 5 Minor breast surgery 10
Study 2 Clonidine (n=15)	49 (29–61)	na	na	12/3	Laparoscopic cholecystectomy
Control (n=15)	38 (27–59)	na	na	13/2	
Study 3 Non-smokers (n=16)	50 (27–58)	16/0	0	12/4	Laparotomy 9 Laparoscopy 5 Vaginal 2
Smokers (n=17)	46 (35–59)	0/17	13	9/8	Laparotomy 3 Laparoscopy 12 Vaginal 2
Study 4 Non-smokers (n=25)	51 (19–68)	25/0	0	17/8	Laparotomy 11 Laparoscopy 11 Vaginal 3
Smokers (n=25)	45 (28–68)	0/25	23	19/6	Laparotomy 13 Laparoscopy 10 Vaginal 2

ASA = American Society of Anesthesiologists physical status, na = not available.

Table 6. Anesthesiological and surgical data, and baseline S-crea and -urea. Data are median (minimum-maximum).

	Duration of anesthesia (min)	Duration of surgery (min)	Blood loss (mL)	Fluid therapy (mL kg^{-1})	MAC-hours
Study 1					
Ketorolac (n = 15)	210 (125–250)	146 (50–207)	400 * (200–1300)	63 (42–88)	3.2 (2.0–4.6)
Control (n = 15)	210 (115–280)	130 (40-225)	200 (50–650)	68 (44–105)	3.4 (1.6–5.4)
Study 2					
Clonidine (n = 15)	95 (50–150)	75 (30–130)	40 (20–60)	32 (22–42)	1.6 (0.8–2.5)
Control (n = 15)	105 (80–230)	85 (60–210)	50 (20–70)	29 (25v45)	1.8 (1.3–3.8)
Study 3					
Non-smokers (n = 16)	93 (60–160)	67 (45–130)	200 (50–350)	31 (27–45)	1
Smokers (n = 17)	100 (50–200)	85 (35–175)	100 (50–500)	33 (24–47)	1
Study 4					
Non-smokers (n = 25)	110 (70–235)	90 (50–210)	100 (50–450)	46 (29–69)	1
Smokers (n = 25)	135 (50–260)	103 (35–240)	100 (20–1500)	50 (31–71)	1

* p = 0.004 (Mann Whitney U-test) between the two study groups. Fluid therapy = Fluids administered during anesthesia and 24 hours after anesthesia, MAC = Minimum alveolar concentration, crea = creatinine, S = serum, na = not available.

GLOMERULAR FUNCTION

SERUM CREATININE AND UREA

None of the 143 patients (studies 1–4) developed acute renal deterioration, seen as increased S-crea or S-urea (table 7). The median of baseline S-crea was 74 (range 47–99) μmol L^{-1} and it decreased to 69 (47–95) μmol L^{-1} at 24 hours after the anesthesia (p = 0.001, Wilcoxon signed ranks test). The highest S-crea, 99 μmol L^{-1}, was measured from a 42-year old man in the clonidine study (study 2).

The median of baseline S-urea was 4.2 (2.3–7.9) mmol L^{-1} and it decreased to 3.1 (1.6–6.6) mmol L^{-1} at 24 hours after the anesthesia (p = 0.001,Wilcoxon signed ranks test). The highest S-urea, 7.9 mmol L^{-1}, was measured from a 59-year old man in the clonidine study (study 2).

SERUM CYSTATIN C

Ketorolac study (study 1)

In study 1, the effect of ketorolac on renal glomerular function was evaluated with S-cystatin C in 20 women undergoing breast surgery with a median of 3.3 MAC-hour sevoflurane anesthesia.

Ketorolac and the sevoflurane anesthesia did not induce glomerular dysfunction and there were no differences in S-cystatin C between the patients with ketorolac and controls ($p = 0.35$). At baseline in the control patients, the median of S-cystatin C was 0.8 (0.6–0.9) mg L^{-1} and at 48 hours after the anesthesia 0.8 (0.5–0.9) mg L^{-1} ($p = 0.21$) and in the patients with ketorolac at baseline 0.8 (0.6–0.9) mg L^{-1} and at 48 hours 0.7 (0.5–0.8) mg L^{-1} ($p = 0.66$), respectively (table 7).

Table 7. Baseline and postoperative S-crea, -urea and -cystatin C. Data are median (minimum-maximum).

	Baseline S-crea (μmol L^{-1})	Postoperative S-crea (μmol L^{-1})	Baseline S-urea (mmol L^{-1})	Postoperative S-urea (mmol L^{-1})	Baseline S-cystatin C (mg L^{-1})	Postoperative S-cystatin C (mg L^{-1})
Study 1 Ketorolac (n = 15)	74 (51–93)	68 (48–76)	4.4 (3.5–5.5)	3.0 (2.0–4.0)	0.8 (0.6–0.9)	0.7 (0.5–0.8)
Control (n = 15)	77 (53–95)	63 (47–88)	4.3 (2.7–6.5)	3.1 (2.2–3.7)	0.8 (0.6–0.9)	0.8 (0.5–0.9)
Study 2 Clonidine (n = 15)	72 (47–95)	66 (55–95)	4.0 (2.3–7.9)	3.1 (1.8–6.6)	na	na
Control (n = 15)	75 (54–99)	73 (54–87)	4.1 (2.5–7.5)	2.8 (1.7–5.7)	na	na
Study 3 Non-smokers (n=16)	78 (65–90)	70 (57–87)	na	na	na	na
Smokers (n=17)	74 (54–87)	73 (58–86)	na	na	na	na
Study 4 Non-smokers (n=25)	74 (63–96)	68 (53–87)	na	na	na	na
Smokers (n=25)	69 (54–87)	69 (57–92)	na	na	na	na

na = not available, crea = creatinine, S = serum

SERUM AND URINE TATI

Enflurane study (study 3)

In studies 3 and 4, S- and U-TATI were used to assess the renal glomerular function.

Serum TATI did not change after one MAC-hour enflurane inhalation, and there were no differences in S-TATI between the two study groups ($p = 0.23$). In the non-smokers, the median of S-TATI at baseline was 1.0 (0.5–1.6) nmol L^{-1} and at 24 hours 1.0 (0.5–2.8) nmol L^{-1} ($p = 0.35$), and in the smokers at baseline 1.3 (0.5–5.2) nmol L^{-1} and at 24 hours 1.3 (0.5–5.8) nmol L^{-1} ($p = 1.0$). At baseline S-TATI was over 3 nmol L^{-1} in 2/17 smokers. After the enflurane anesthesia, S-TATI increased over 2 nmol L^{-1} in one patient and over 3 nmol L^{-1} in two patients in both study groups. The patients with high S-TATI did not have any higher $S\text{-}F^-$, median 14 (range 13–23) μmol L^{-1}, than those with S-TATI within the reference values, $S\text{-}F^-$ 16 (8.4–38) μmol L^{-1} ($p = 0.83$).

There were no differences in U-TATI at baseline or after one MAC-hour enflurane anesthesia between the two study groups ($p = 0.38$). However, U-TATI increased both in the non-smokers, the median of U-TATI/crea 0.4 (0.1–1.4) 10^{-6} at baseline to 0.5 (0.1–2.7) 10^{-6} at 24 hours after the anesthesia ($p = 0.021$, Wilcoxon signed ranks test), and in the smokers U-TATI/crea 0.6 (0.1–5.1) 10^{-6} at baseline increased to 0.7 (0.2–7.3) 10^{-6} at 24 hours ($p = 0.009$). At 48 hours the median of U-TATI/crea decreased to baseline both in the non-smoking patients, 0.6 (0.3–3.4) 10^{-6} and in smoking patients, 0.5 (0.3–1.6) 10^{-6}. At baseline U-TATI/crea was over 0.64 10^{-6} in 4/16 non-smoking and in 5/17 smoking patients. At 24 hours U-TATI/crea increased to over 0.64 10^{-6} in 1/16 non-smokers and in 5/17 smokers ($p=0.085$).

Sevoflurane study (study 4)

In study 4, S-TATI increased after sevoflurane anesthesia in both the non-smokers and the smokers and there were no differences in S-TATI between the two study groups at baseline or at 24 hours after the sevoflurane inhalation ($p = 0.31$). In the non-smokers, the median of S-TATI 1.3 (0.5–2.1) nmol L^{-1} at baseline increased to 1.4 (0.5–3.1) nmol L^{-1} at 24 hours after the anesthesia ($p = 0.031$, Wilcoxon signed ranks test). In the smokers, S-TATI 1.3 (0.5–2.6) nmol L^{-1} at baseline increased to 1.6 (0.5–18) nmol L^{-1} at 24 hours after the anesthesia ($p = 0.005$).

S-TATI increased above the upper concentration of normal of 2 nmol L^{-1} in 5/25 non-smokers and in 12/25 smokers ($p = 0.035$, chi square test) after one MAC-hour sevoflurane anesthesia. Serum TATI increased above the pathological concentration of 3.0 nmol L^{-1} in 11 women, (four non-smokers and seven smokers, $p = 0.4$). In a post-hoc analysis it was revealed that in all five women (one non-smoker and four smokers) with $S\text{-}F^-$ over 40 μmol L^{-1} and the 24 hour area under $S\text{-}F^-$ concentration time curve ($AUCF_{0\text{-}24}$) over 500 μmol h L^{-1} S-TATI increased over 3.0 nmol L^{-1}. On the contrary, among the 45 women with the maximum $S\text{-}F^-$ less than 40 μmol L^{-1}, only six had S-TATI over 3.0 nmol L^{-1} ($p = 0.001$).

In four women, one non-smoker and three smokers, with both increased S-TATI (≥ 3.0 nmol L^{-1}) and $S\text{-}F^-$ (≥ 40 μmol L^{-1}) U-TATI/crea increased over 0.64 10^{-6}. Confounding factors to be taken into account when using S-TATI for assessment of glomerular function are presented in study 4.

SERUM β2-MICROGLOBULIN

Ketorolac study (study 1)

The effect of ketorolac on renal glomerular function was evaluated with S-β2-MG in patients with a 3.3 MAC-hour sevoflurane anesthesia.

In that study, S-β2-MG remained at low concentrations. Even the highest S-β2-MG measured, 1.9 mg L^{-1} from a control patient at 48 hours after the anesthesia, S-β2-MG was within the reference limits of 0.6–3.0 mg L^{-1} (Hospital district of Helsinki and Uusimaa, Huslab 2005).

Enflurane (study 3) and sevoflurane study (study 4)

In study 3 and study 4 the effects of $S\text{-}F^{-}$ on glomerular function were evaluated in the non-smokers and the smokers undergoing gynecological surgery with one MAC-hour enflurane or sevoflurane anesthesia.

In both studies, S-β2-MG remained below 3.0 mg L^{-1} in all patients during the whole study period.

PROXIMAL TUBULUS

The effect of sevoflurane anesthesia with ketorolac (study 1) on proximal tubule function was evaluated with U-α1-MG/crea-ratio. The effect of inhalation anesthesia on cellular integrity of proximal tubules was evaluated in the non-smoking and smoking patients, and in the patients with ketorolac and with clonidine using U-NAG/crea (study 1, 2), U-β2-MG (study 1, 3, 4), and U-GST-α/crea-ratio (study 1). The results of tubular function and cellular damage biomarkers that are not correlated to U-crea are presented in tables 8 and 9 (studies 1, 2).

URINE α1-MICROGLOBULIN

Ketorolac study (study 1)

In study 1, U-α1-MG/crea was not a reliable indicator of the proximal tubular dysfunction. In this population U-α1-MG/crea was > 0.7 g mol^{-1} (the highest ratio measured from healthy volunteers, Teppo et al. 2000) already at baseline in 4/15 controls and in 2/15 patients with ketorolac ($p = 0.65$).

In the controls, U-α1-MG/crea increased to its highest at 48 hours ($p = 0.061$). In the control patients U-α1-MG/crea, 1.2 (0.1–3.1) g mol^{-1}, at 48 hours, was significantly higher than that in the patients with ketorolac, 0.4 (0.1–3.1) g mol^{-1}, (mean difference 0.6 g mol^{-1}, 95% confidence interval (CI) for difference: 0.03 to 1.2 g mol^{-1}, $p = 0.021$, Mann Whitney U-test) (Figure 6). In all patients except one with ketorolac U-α1-MG/crea was higher than > 0.7 g mol^{-1} at some state of surgery or the 48 hours recovery period, and at 48 hours 11/15 controls and 5/15 with ketorolac still had U-α1-MG/crea > 0.7 g mol^{-1} ($p = 0.028$).

URINE N-ACETYL-β-D-GLUCOSAMINIDASE

Ketorolac study (study 1)

In study 1, an increase in U-NAG/crea, a marker of proximal tubular deterioration, was noted in both the controls and the patients with ketorolac undergoing breast surgery with a 3.3 MAC-hour sevoflurane anesthesia ($p = 0.001$ Friedman test). In the controls, the median of U-NAG/crea increased from 1.3 (0.2–21) units g^{-1} at baseline to 3.5 (0.7–13) units g^{-1} at two hours of anesthesia and in the patients with ketorolac the median of U-NAG/crea increased from 1.1 (0.5–3.8) units g^{-1} at baseline to 3.0 (1.5–17) units g^{-1} at two hours in anesthesia, ($p = 0.015$, Wilcoxon signed ranks test). Urine NAG/crea returned to the baseline at 12 hours after the anesthesia in both groups.

At baseline the uncorrelated U-NAG was over 6.1 units L^{-1} (above the highest concentration measured from healthy volunteers) in one control and in one patient with ketorolac. During or within 2 hours after the sevoflurane anesthesia, U-NAG increase to over 6.1 units L^{-1} in 5/15 patients with ketorolac, but in none of the controls ($p = 0.042$, Fisher's exact test) (table 8).

Clonidine study (study 2)

Patients with clonidine developed a minor and transient increase in U-NAG/crea during pneumoperitoneum. Urine NAG/crea increased to its highest level at 60 minutes of pneumoperitoneum both in the controls and in the patients with clonidine ($p = 0.001$, Wilcoxon signed ranks test). In the control patients, the median of U-NAG/crea 0.3 (0.2–0.5) units g^{-1} at baseline increased to 0.7 (0.3–1.7) units g^{-1} at 60 minutes of pneumoperitoneum, and in the patients with clonidine U-NAG/crea 0.3 (0.1–0.9) units g^{-1} at baseline increased to 1.1 (0.3–2.4) units g^{-1} at 60 minutes of pneumoperitoneum (mean difference at 60 minutes between the groups 0.5 units g^{-1}, 95% CI for difference: 0.1 to 0.8 units g^{-1}, $p = 0.01$, Mann Whitney U-test). The increase in U-NAG/crea was transient in both groups, and at 3 hours after pneumoperitoneum the concentrations were comparable to those observed at baseline (Figure 7).

At baseline, before the study drug administration, the uncorrelated U-NAG was over 6.1 units L^{-1} (the highest concentration measured from healthy volunteers, Koivusalo 1997) in two controls and one patient with clonidine. During the study period U-NAG increase over 6.1 units L^{-1} in 7/15 controls and in 11/15 patients with clonidine ($p = 0.14$) (table 9).

URINE β2-MICROGLOBULIN

The effects of ketorolac (study 1) and smoking (studies 3 and 4) on renal proximal tubular deterioration were evaluated with U-β2-MG in the patients with sevoflurane (studies 1 and 4) and enflurane anesthesia (study 3).

Ketorolac study (study 1)

In study 1, U-β2-MG remained low without differences between the two groups. High U-β2-MG, > 0.25 mg L^{-1} (Hospital district of Helsinki and Uusimaa, Huslab 2005), at baseline was noted in one patient with ketorolac, and during anesthesia and recovery it increased > 0.25 mg L^{-1} in 2/15 controls and in 4/15 patients with ketorolac.

In study 1, no differences were noted in U-β2-MG between the non-smokers and smokers ($p = 0.4$).

Enflurane study (study 3)

In study 3, U-β2-MG remained low without differences between the two study groups (at baseline $p = 0.6$, at 24 hours $p = 0.16$).

Sevoflurane study (study 4)

In study 4, the median of U-β2-MG remained low in both study groups.

Urine β2-MG increased markedly (> 1 mg L^{-1}) in 2/25 non-smokers and in 2/25 smokers after sevoflurane inhalation. In a post-hoc analysis it was revealed that U-β2-MG increased significantly in 2/5 patients with $S\text{-}F^-$ over 40 μmol L^{-1} compared to 2/45 patients with the highest $S\text{-}F^-$ less than 40 μmol L^{-1} ($p = 0.005$, chi-square test).

URINE GLUTATHIONE TRANSFERASE-α

Ketorolac study (study 1)

Urine GST-α/crea increased in the patients with sevoflurane anesthesia. At baseline there was no differences between the two groups in the median of U-GST-α/crea ($p = 0.9$). Urine GST-α/crea increased to its highest in both groups at two hours after sevoflurane anesthesia ($p = 0.001$, Wilcoxon signed ranks test). However, after anesthesia it was significantly lower in the controls, 0.4 (0.1–3.0) mg mol^{-1}, than that in the patients with ketorolac, 1.5 (0.1–5.9) mg mol^{-1}, (mean difference 1.0 mg mol^{-1}, 95% CI for difference: 0.02 to 2.0 mg mol^{-1}, $p = 0.016$, Mann-Whitney U-test). Urine GST-α/crea increased over 1.9 mg mol^{-1}, the highest ratio observed from healthy volunteers (Teppo A-M, personal communication 2000), in 3/15 controls and in 7/15 patients with ketorolac ($p = 0.068$) (table 8).

DISTAL TUBULUS

URINE GLUTATHIONE TRANSFERASE-π

Ketorolac study (study1)

In study 1, the effects of ketorolac on distal tubular cellular integrity were evaluated with U-GST-π/crea in the patients with a 3.3 MAC-hour sevoflurane anesthesia.

Urine GST-π/crea increased similarly in both study groups to its maximum at two hours after the anesthesia; in the control patients the median of U-GST-π/crea 0.1 (0.04–0.5) mg mol^{-1} at baseline increased to 0.8 (0.1–9.5) mg mol^{-1} at two hours after the anesthesia ($p = 0.001$, Wilcoxon signed ranks test), and in the patients with ketorolac from 0.1 (0.05–1.2) mg mol^{-1} at baseline to 1.3 (0.4–6.1) mg mol^{-1} at two hours ($p = 0.001$, Wilcoxon signed ranks test). Urine GST-π/crea increased above 7.4 mg mol^{-1} in two control patients ($p = 0.48$).

KETOROLAC

KETOROLAC AND BLEEDING

In study 1, the patients underwent mastectomy and axillary dissection (n = 9) or minor breast surgery (n= 21). Sevoflurane was used for anesthesia maintenance with a median exposure of 3.3 MAC-hour.

The surgical bleeding was twice as low in the controls, 200 (50–650) mL (median (minimum–maximum)), compared to the patients with ketorolac, 400 (200–1300) mL ($p = 0.004$, Mann-Whitney U-test). The most abundant blood loss was in 2/15 patients with ketorolac, 800 mL and 1300 mL respectively, and packed red blood cells were administered in these two patients. No reoperations due to bleeding were performed. There was no difference in blood hemoglobin concentration and hematocrit between the two study groups at baseline or at 24 hours after surgery.

KETOROLAC AND FLUORIDE

In study 1, no differences in F^- excretion between the two study groups were noted; in the control patients the $AUCF_{0\text{-}48}$ was 630 (160–810) μmol h L^{-1} (median (minimum-maximum)) and in the patients with ketorolac 540 (280–1200) μmol h L^{-1} ($p = 0.87$).

Serum F^- increased to the maximum at two hours after sevoflurane anesthesia both in the controls, from 4.2 (2.6–4.6) μmol L^{-1} at baseline to 32 (18–47) μmol L^{-1}, ($p = 0.001$, Wilcoxon signed ranks test), and in the patients with ketorolac, from 4.0 (1.4–6.4) μmol L^{-1} to 26 (21–50) μmol L^{-1}, ($p = 0.001$). 4/15 controls and 3/15 patients with ketorolac had S- F^- 40 μmol L^{-1} or higher at two hours after sevoflurane anesthesia. Serum F^- remained elevated until 48 hours after sevoflurane anesthesia and no differences between the two study groups were noted (Figure 9).

Ketorolac had no effect on U-F^-. In the control patients U-F^- increased from 48 (33–100) μmol L^{-1} at baseline to the highest of 820 (150–3400) μmol L^{-1} and in the patients with ketorolac from 50 (24–120) μmol L^{-1} to 1900 (380–4200) μmol L^{-1} at 12 hours after anesthesia. The urine volumes were similar in both study groups.

CLONIDINE, PNEUMOPERITONEUM AND RENAL FUNCTION

In study 2, the S-ADH and P-RA were evaluated as factors possibly affecting renal function during pneumoperitoneum for laparoscopic cholecystectomy.

No differences in S-ADH were noted between the two study groups. At baseline, in the control patients, the median of S-ADH was 4.0 (0.4–26) ng L^{-1}, and it increased to 40 (1.0–220) ng L^{-1} at 60 minutes of pneumoperitoneum ($p = 0.001$, Wilcoxon signed ranks test). A similar increase was noted in the patients with clonidine, S-ADH 3.0 (0.3–13) ng L^{-1} at baseline increased to 67 (1.0–280) ng L^{-1} at 60 minutes of pneumoperitoneum ($p = 0.003$).

The median of urine output in the controls was 0.6 (0.1–3.2) mL kg^{-1} h^{-1} and in the patients with clonidine 1.5 (0.5–4.0) mL kg^{-1} h^{-1} (p = 0.061). 8/15 controls and 1/15 with clonidine had urine output 0.5 mL kg^{-1} h^{-1} or less (p = 0.005, chi square test). In the controls P-RA at 60 minutes of pneumoperitoneum and at 60 minutes after surgery correlated inversely with urine output at 120 minutes after pneumoperitoneum (Pearson correlation coefficient: –0.526, p = 0.044, and –0.623, p = 0.01, respectively).

In study 2, the clonidine-treated patients had low concentrations of P-RA during pneumoperitoneum. In the controls, the median of P-RA at anesthesia induction (60 minutes after the study drug injection) was 2.3 (0.2–5.4) ng L^{-1} and in the patients with clonidine 0.8 (0.4–3.2) ng L^{-1} (p = 0.07). At 15 minutes of pneumoperitoneum P-RA was in the control patients 5.1 (0.4–11) ng L^{-1} and in the patients with clonidine 1.3 (0.5–5.7) ng L^{-1} (mean difference 3.1 ng L^{-1}, 95% CI for difference: 1.0 to 5.3 ng L^{-1}, p = 0.033, Mann Whitney U-test). Also, P-RA was higher in the controls after 30 minutes of pneumoperitoneum, 5.0 (0.3–14) ng L^{-1}, than in the patients with clonidine 1.7 (0.2–7.0) ng L^{-1} (mean difference 3.5 ng L^{-1}, 95% CI for difference: 1.0 to 5.9 ng L^{-1}, p = 0.026).

EFFECT OF SMOKING ON THE METABOLISM OF ENFLURANE AND SEVOFLURANE

ENFLURANE STUDY (study 3)

In study 3 the effects of smoking on $S\text{-}F^-$ production after one MAC-hour enflurane anesthesia were evaluated in 33 women undergoing gynecological surgery.

In the non-smoking group, the median of $AUCF_{0\text{-}24}$ was 220 (130–350) μmol h L^{-1} and in the smokers the median of $AUCF_{0\text{-}24}$ was between 320 (120–620) μmol h L^{-1}, (the mean difference 101 μmol h L^{-1}, 95% CI for difference: 12 to 190 μmol h L^{-1}, p = 0.028, Mann Whitney U-test) after one MAC-hour enflurane anesthesia. The detected $S\text{-}F^-$ had its highest concentration at six hours after the anesthesia in both groups; in the non-smokers $S\text{-}F^-$ increased to 12 (6.6–21) μmol L^{-1} (p = 0.001, Wilcoxon signed ranks test) and in the smokers to 18 (7.5–38) μmol L^{-1} (p = 0.001) (Figure 9).

SEVOFLURANE STUDY (study 4)

In the sevoflurane study (study 4) the effects of smoking on $S\text{-}F^-$ production was evaluated after one MAC-hour sevoflurane anesthesia in 50 women undergoing gynecological surgery.

In contrast to enflurane, smoking did not affect the F^- production after sevoflurane anesthesia. In the non-smokers the median of $AUCF_{0\text{-}24}$ was 320 (38–710) μmol h L^{-1} and in the smokers 320 (130–970) μmol h L^{-1} (the mean difference 55 μmol h L^{-1}, 95% CI for difference: –42 to 150 μmol h L^{-1}). The individual $AUCF_{0\text{-}24}$ in the non-smoking and smoking patients with sevoflurane are presented in figure 8 a and b.

The $S\text{-}F^-$ peaked at two hours after sevoflurane anesthesia in both groups; in the non-smokers $S\text{-}F^-$ increased from 1.6 (1.0–11) μmol L^{-1} at baseline to 26 (8.2–40) μmol L^{-1} at two hours after

anesthesia (p = 0.0001, Wilcoxon signed ranks test) and in the smokers from 1.7 (0.5–5.2) μmol L^{-1} to 25 (17–71) μmol L^{-1} (p = 0.0001,) (Figure 10). 1/25 non-smokers and 4/25 smokers had $S\text{-}F^-$ 40 μmol L^{-1} or higher.

KETOROLAC STUDY (study 1)

The primary aim of study 1 was not to evaluate the effects of smoking on sevoflurane metabolism and F^- production, but because both smokers (n=10) and non-smokers (n=20) were included, in a post-hoc analysis, these two populations were compared.

In accordance with the sevoflurane study (study 4), there were no differences in F^- production between the non-smokers, $AUCF_{0\text{-}48}$ 580 (160–1200) μmol h L^{-1}, and the smokers, $AUCF_{0\text{-}48}$ 600 (260–1200) μmol h L^{-1}. 3/20 non-smoking patients and 4/10 smoking patients had the highest $S\text{-}F^-$ 40 μmol L^{-1} or higher (p = 0.13).

Although the depicted F^- release after the 3.3 MAC-hour sevoflurane anesthesia, $AUCF_{0\text{-}24}$ 440 (160–860) μmol h L^{-1}, exceeded that after one MAC-hour sevoflurane anesthesia, 320 (380–970) μmol h L^{-1} (mean difference 99 μmol h L^{-1}, 95% CI for difference: 22 to 180 μmol h L^{-1}, Mann-Whitney U-test p = 0.003), F^- release was not dose proportional. Fluoride production was only 1.4 fold higher despite 3.3 time higher sevoflurane exposure.

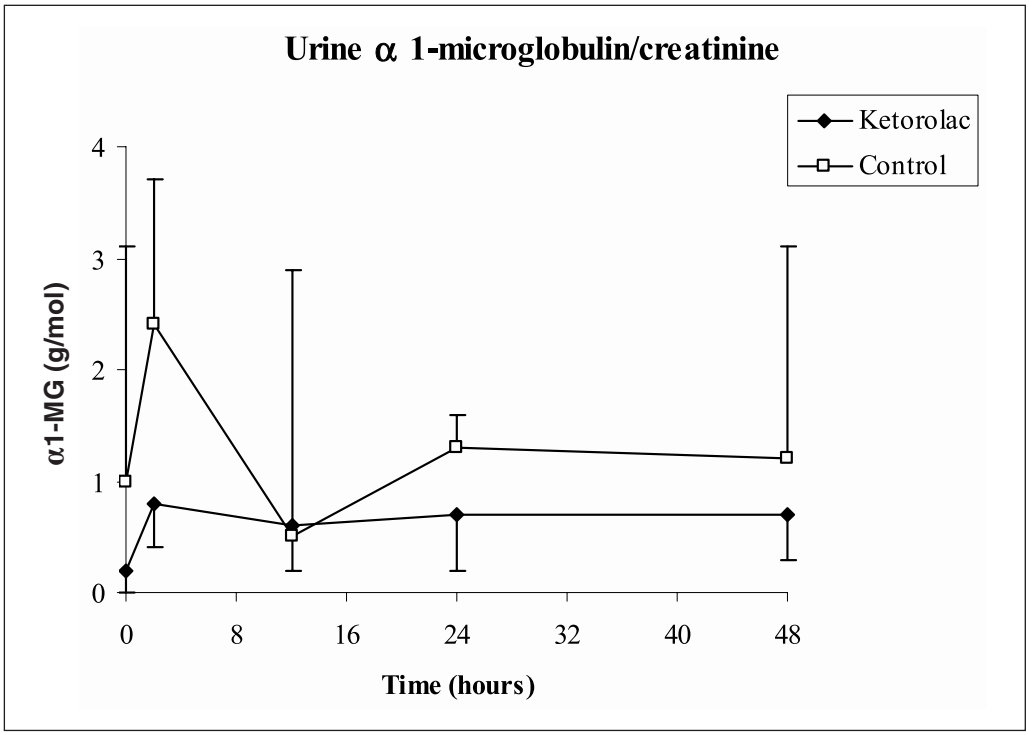


Figure 6. Urine α1-MG/crea in patients with ketorolac undergoing breast surgery (study 1, publication II). Means and standard deviations are depicted.

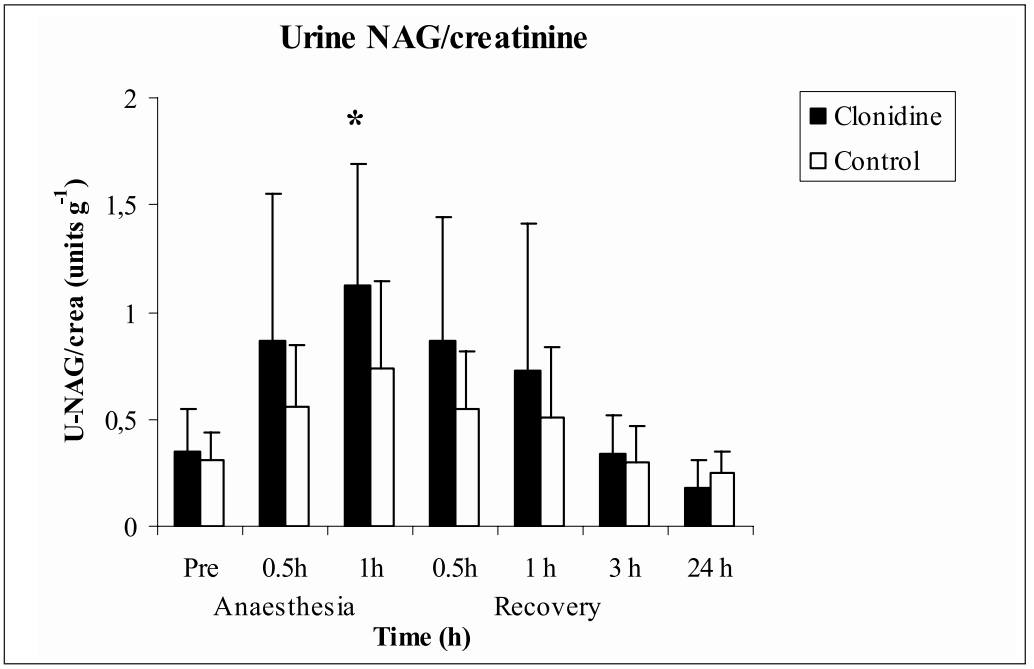


Figure 7. Urine NAG/crea in the patients with clonidine undergoing laparoscopic cholecystectomy (study 2). Data are represented as means and standard deviations. * p = 0.01 (Mann.Whitney U-test).

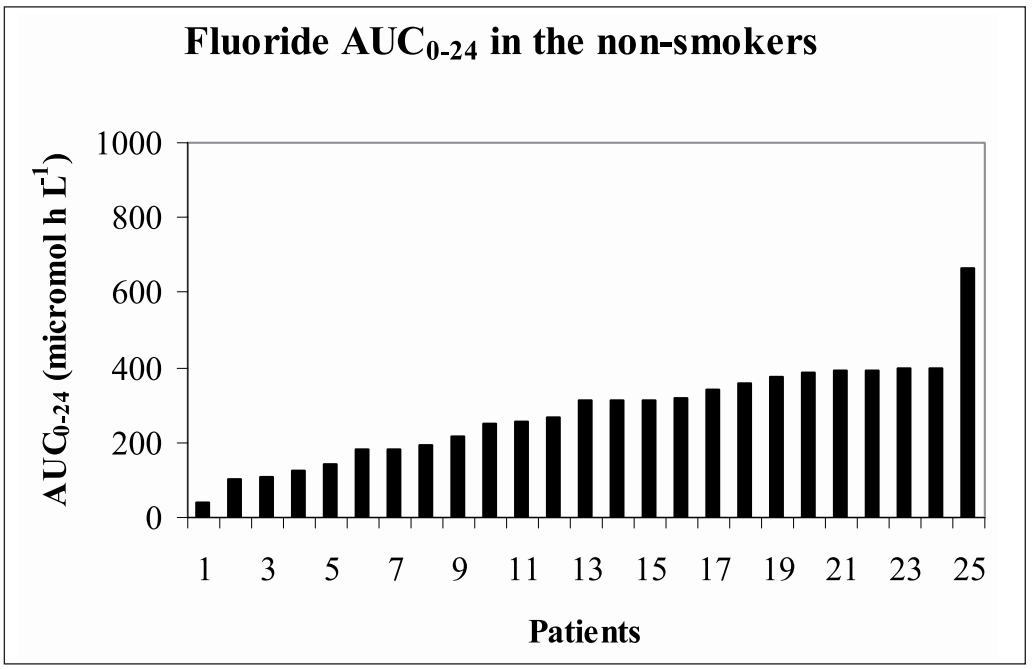


Figure 8a. The serum $AUCF_{0\text{-}24}$ of inorganic fluoride (μmol h L^{-1}) from the lowest to the highest value in the non-smoking patients (n = 25) after one MAC-hour sevoflurane anesthesia.

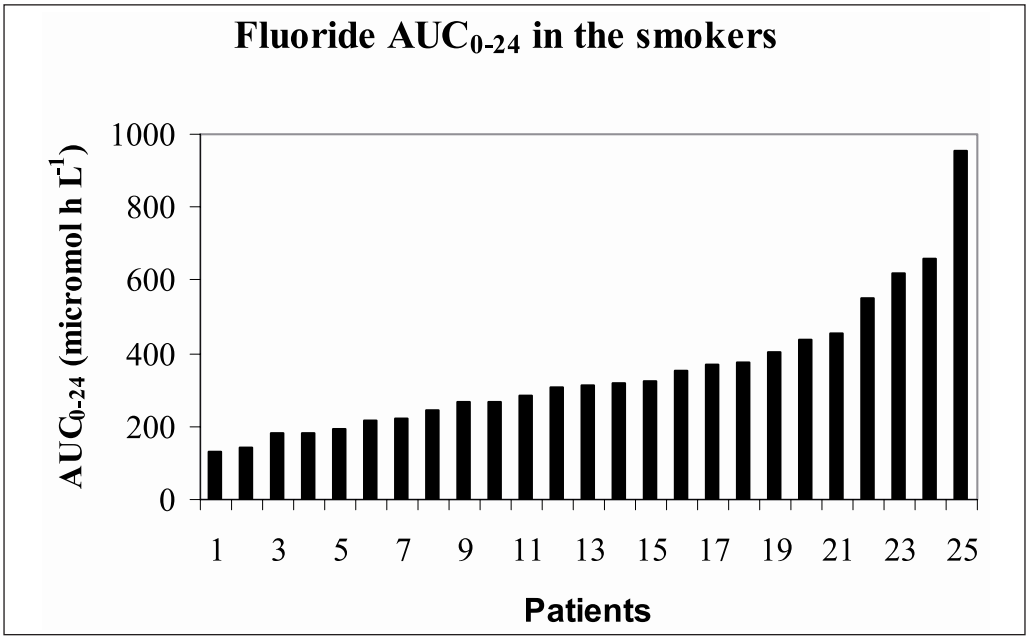


Figure 8b. The serum $AUCF_{0\text{-}24}$ of inorganic fluoride (μmol h L^{-1}) from the lowest to the highest value in the smoking patients (n = 25) after one MAC-hour sevoflurane anesthesia.

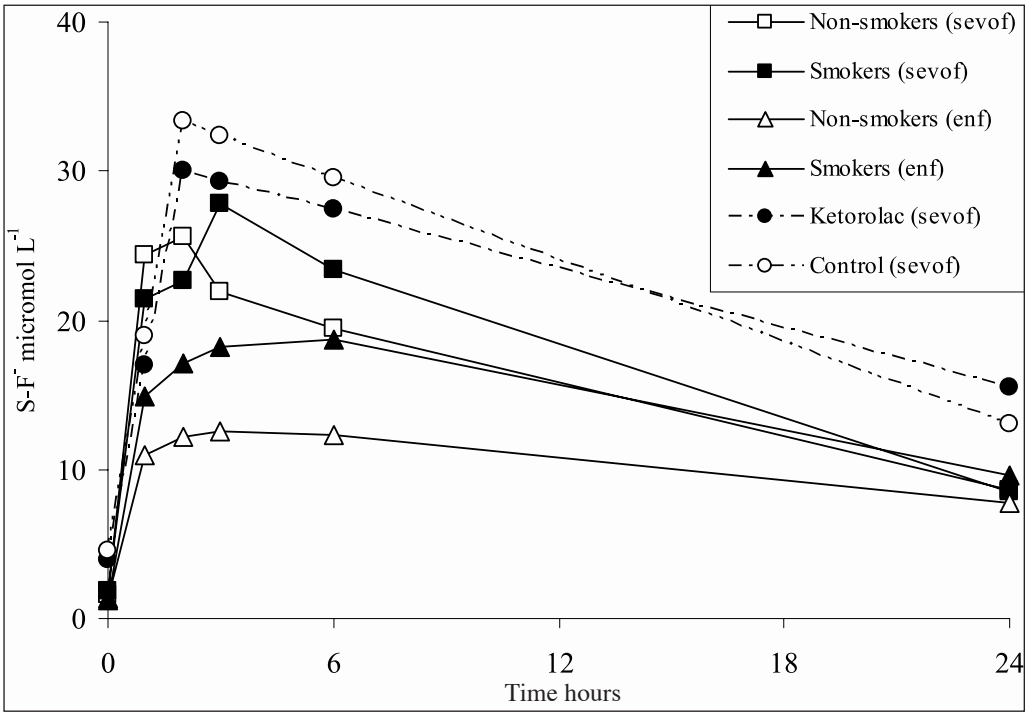


Figure 9. Serum fluoride concentrations (μmol L^{-1}), in patients after 1 MAC-hour sevoflurane and enflurane anesthesia (non-smokers and smokers) and after 3.3 MAC-hour sevoflurane anesthesia in patients with and without ketorolac (control), data are means.

Table 8. Urine renal biomarkers in the patients with and without ketorolac after 3.3. MAC-hour sevoflurane anesthesia. Values are not correlated to urine creatinine, data are median and (minimum-maximum) (study 1).

	Baseline	**120 min**	**2 h after anesthesia**	**12 h after anesthesia**	**24 h after anesthesia**	**48 h after anesthesia**
U-crea (g L^{-1})						
Ketorolac	1.3 (0.2–2.6)	0.8 (0.2–2.6)	0.9 (0.1–1.9)	1.0 (0.1–2.0)	0.6 (0.1–2.1)	0.6 (0.1–1.6)
Controls	1.0 (0.2–2.6)	0.4 (0.1–1.4)	0.6 (0.1–2.0)	0.6 (0.1–1.9)	0.3 (0.1–1.0)	0.8 (0.2–1.5)
U-α1-MG (mg L^{-1})						
Ketorolac	1.3 (0.5–17.0)	5.1 (0.7–14.4)	5.6 (0.8–14.4)	5.2 (0.8–17.4)	2.6 (0.6–15.5)	2.2 (0.7–10.0)
Controls	2.0 (0.4–18.4)	1.1 (0.7–13.3)	5.3 (0.7–14.4)	1.8 (0.4–13.8)	2.6 (0.1–6.9)	8.3 ** (0.6–23.1)
U-NAG (units L^{-1})						
Ketorolac	1.1 (0.5–12.0)	3.0 (1.0–12.4)	2.2 (0.1–12.2)	1.4 (0.1–6.2)	1.2 (0.4–3.6)	0.6 (0.1–3.0)
Controls	0.9 (0.2–5.0)	2.3 (0.3–7.5)	1.9 (0.4–10.3)	0.5 (0.1–4.3)	0.2 (0.1–1.0)	1.5 (0.1–4.7)
U-GST-α (μg L^{-1})						
Ketorolac	0.9 (0.9–3.2)	2.0 (0.9–27.5)##	8.5 (0.9–61.0)##	1.5 (0.9–8.0)#	0.9 (0.9–3.5)	0.9 (0.9–3.5)
Controls	0.9 (0.9–4.0)	1.5 (0.9–4.5)	1.4 (0.9–18.0)	1.3 (0.9–15)	0.9 (0.9–2.0) #	1.4 (0.9–2.2)
U-GST-π (μg L^{-1})						
Ketorolac	0.9 (0.9–2.2)	2.0 (0.9–43.0)#	14.1 (0.9–45.0)##	1.6 (0.9–3.6)	0.9 (0.9–8.1)	0.9 (0.9–4.0)
Controls	0.9 (0.9–2.5)	2.0 (0.9–37.5)#	2.2 (0.9–48.5#)	1.0 (0.9–3.8)	1.1 (0.9–1.7)	3.2 (0.9–8.5)#

* $p<0.05$, ** $p<0.01$ between the study groups (Mann-Whitney U-test), # $p<0.05$, ## $p<0.01$ comparing to the baseline concentration within the study group (Wilcoxon signed ranks test). U = urine, crea = creatinine, α1-MG = α1-microglobulin, GST-α = Glutathione-S-transferase -α, GST-π = Glutathione-S-transferase -π.

Table 9. Urine N-acetyl-β-D-glucosaminidase and creatinine in patients with and without clonidine during laparoscopic cholecystectomy. Values are not correlated to urine creatinine. Data are median and (minimum-maximum) (study 2).

	Baseline	**30 min**	**60 min**	**30 min after PP**	**1h after PP**	**3h after PP**	**24 h after PP**
U-NAG (units L^{-1})							
Clonidine	2.6 (0.4–6.8)	2.9 (0.7–7.9)	4.0 (0.1–12.4)	4.8 (1.0–28.6)	2.2 (1.0–25.1)	1.8 (0.8–18.1)	1.3 (0.6–5.3)
Controls	2.2 (0.4–7.0)	2.7 (0.3–8.2)	3.9 (0.1–15.7)	3.6 (0.4–14.6)	3.4 (0.1–9.5)	1.9 (0.3–4.5)	1.8 (0.2–5.6)
U-crea (g L^{-1})							
Clonidine	11.1 (1.4–21.4)	3.1 (0.1–20.3)#	4.1 (0.1–14.4)#	6.9 (0.1–16.8)	6.3 (0.1–25.4)	7.0 (1.9–30.7)	9.1 (3.0–31.0)
Controls	9.7 (1.6–19.7)	4.8 (1.1–21.0)	6.2 (0.1–17.3)	8.4 (0.1–22.2)#	7.9 (0.1–15.6)	7.3 (1.4–16.9)	8.1 (4.0–20.2)

# $p<0.05$ compared to the baseline within the study group. 30 min = 30 minutes of pneumoperitoneum, 60 min = 60 minutes of pneumoperitoneum, crea = creatinine, PP = pneumoperitoneum, U = urine, NAG = N-acetyl-β-D-glucosaminidase.

DISCUSSION

KETOROLAC

GLOMERULAR FILTRATION

Co-administration of ketorolac with 3.3 MAC-hour sevoflurane anesthesia does not seem to decrease glomerular filtration in surgical patients. In the present study S-crea, S-cystatin C and S-β2-MG did not increase in either the patients with or without ketorolac after sevoflurane anesthesia.

Contradictory findings in the effects of NSAIDs on renal function in surgical patients have been presented. In previous studies, a decrease in crea clearance (Freedland et al. 2002, Lee et al. 2004) has been shown in patients with perioperative NSAID. However, crea clearance calculated with the Cockcroft-Gault equation (Cockcroft and Gault 1976) used by Freedland and co-workers (2002) is susceptible to errors from patients' age, gender and weight (Verhave et al. 2003). Serum cystatin C and S-β2-MG measurements used in the present study are more sensitive and reliable markers of glomerular function and less affected by the patient characteristics (Harmoinen 2001, Harmoinen et al. 2003).

There are some differences in the study designs, which may explain the contradictory results. The dosage of ketorolac (1.8 mg kg^{-1}) was higher in the Freedland and co-workers (2002) study than that in the present study (1.3 mg kg^{-1}). Moreover, in the Freedland's study the anesthesia and the fluid therapy were not reported, leaving unanswered possible hypotension or hypovoelemia as risk factors for ARF in addition to NSAID (Wilson and Aronson 2001). In the present study, the lower dose of ketorolac and sufficient fluid therapy may have prevented abundant glomerular deterioration in patients undergoing breast surgery with sevoflurane anesthesia.

In summary, ketorolac 90 mg, did not affect renal glomerular function in patients undergoing 3.3 MAC-hour sevoflurane anesthesia.

PROXIMAL TUBULAR FUNCTION

If compared with the reference values obtained from healthy volunteers, U-α1-MG/crea appeared to be an unreliable indicator of the proximal tubular dysfunction in patients having breast surgery. In study 1, U-α1-MG/crea was higher than the upper limit of normal, > 0.7 g mol^{-1} (Teppo et al. 2000), in one fifth of the patients undergoing breast surgery already at baseline. Moreover, U-α1-MG/crea was > 0.7 g mol^{-1} at some state of the perioperative period in almost all patients in the ketorolac study. Therefore, it remains unanswered whether the patients had actually developed a proximal tubular dysfunction or whether the observation was biased due to an unreliable method.

The present study indicates that perioperative ketorolac may not be harmful for proximal tubular function in patients with sevoflurane anesthesia if a sufficient fluid therapy is provided. In the present study, a small increase in U-α1-MG/crea was noted in the controls but no such increase was noted in the patients with ketorolac, and at 48 hours after anesthesia, fewer patients with ketorolac had U-α1-MG/crea > 0.7 g mol^{-1} than controls.

However, further studies are warranted in surgical patients to establish the reference values for U-α1-MG/crea and to establish the reliability of U-α1-MG/crea as a marker of proximal tubular function. The invasiveness of surgery may also affect U-α1-MG/crea excretion. In the present study, the patients had breast surgery and no clinically significant increase in U-α1-MG/crea was noted, whereas, in patients undergoing coronary artery bypass grafting surgery, U-α1-MG increased significantly and remained elevated for several days after surgery (Gormley et al. 2000, Boldt et al. 2003).

In summary, with the reference values obtained from healthy volunteers in surgical patients, U-α1-MG/crea is an unreliable marker of proximal tubular function and reference limits of U-α1-MG/crea are yet to be established in surgical patients.

PROXIMAL TUBULAR CELLULAR DAMAGE

3.3 MAC-hour sevoflurane anesthesia may induce some degree of injury in proximal tubular cells. In the present study U-GST-α/crea and U-NAG/crea increased in all patients having sevoflurane and isoflurane anesthesia. However, the injury caused by sevoflurane may be aggravated by a concomitant administration of ketorolac. After sevoflurane anesthesia (study 1), the median of U-GST-α was significantly higher in the patients with ketorolac than in the controls. Also, the proportion of patients with U-GST-α higher than that obtained from healthy volunteers was greater in the ketorolac group than in the controls (Teppo personal communication 2000).

Also, the uncorrelated U-NAG increased more than that obtained from healthy volunteers, 6.1 units L^{-1}, in 5/15 patients with ketorolac having 3.3 MAC-hour sevoflurane anesthesia but in none of the controls, indicating that during sevoflurane anesthesia ketorolac may have harmful effects on proximal tubular integrity. There were no differences in other biomarkers of proximal tubular damage between the two study groups; U-β2-MG remained at low concentrations during the length of the study period both in the patients with ketorolac and in the controls. Serum F^- does not seem to be the reason for increased U-NAG/crea or U-GST-α/crea, as there was no correlation between these biomarkers and the rise in S-F^-.

Supporting the results of the present study, Higuchi and co-workers (1995) noted an increase in U-NAG/crea after 9–11 MAC hour sevoflurane anesthesia. However, there are some differences between the two studies. Higuchi measured the highest U-NAG/crea concentrations at 48 hours after the surgery compared to maximum U-NAG/crea at two hours in the present study. In the Higuchi-study U-NAG/crea was still elevated 72 hours after surgery while in the present study U-NAG/crea decreased to the baseline at 12 hours after surgery.

In accordance to the present result, a significant increase in U-GST-α was demonstrated at 24 and at 48 hours after 10 MAC-hour sevoflurane anesthesia in a volunteer study (Eger et al. 1997). Contradictory results on inhalation anesthetics effects on sensitive biomarkers of proximal tubular cellular damage have been reported. In Kharasch and co-workers' study (1997), no increase in U-GST-α/crea or U-NAG/crea was found in surgical patients with 4.5 MAC-hour sevoflurane or isoflurane anesthesia. In another study using a similar protocol as Eger et al. (1997), Ebert and co-workers (1998a) did not find significant changes in U-GST-α.

In summary, 3.3 MAC-hour sevoflurane anesthesia may induce minor proximal tubular cellular deterioration, noted as increased urine release of U-NAG/crea and -GST-α/crea in the patients, and perioperative ketorolac administration may enhance the deterioration.

DISTAL TUBULUS

Concomitant use of ketorolac with sevoflurane anesthesia does not seem to affect distal tubular cellular integrity. In study 1, a slight increase in U-GST-π/crea was noted after 3.3 MAC hour sevoflurane anesthesia, but U-GST-π/crea remained below the reference limit of 7.4 mg mol^{-1} (Teppo personal communication 2000) in all except one patient. This is in agreement with Kharasch and co-workers (1997) who did not note increase in U-GST-π/crea in patients with sevoflurane or isoflurane anesthesia. Although Eger and co-workers (1997) reported an increase of U-GST-π in volunteers with sevoflurane anesthesia, Ebert et al. (1998a) did not note such an increase.

In summary, perioperative ketorolac does not seem to augment distal tubular cellular damage in patients with sevoflurane anesthesia.

CLONIDINE

PROXIMAL TUBULUS

Pneumoperitoneum and isoflurane anesthesia causes a transient proximal tubular cellular deterioration and co-administration of clonidine may enhance the damage. In the present study, more patients with clonidine than controls had the uncorrelated U-NAG in excess of the highest reference concentration obtained from healthy volunteers (6.1 units L^{-1}, Koivusalo 1997). However, although U-NAG, which is a sensitive marker of proximal tubular damage, increased during pneumoperitoneum in the patients with isoflurane, U-NAG concentrations were not significantly higher than those observed in the ketorolac study in the patients having sevoflurane anesthesia for breast surgery (study 1).

An IAP exceeding 15 mmHg reduces renal cortical blood flow by 40% and decreases GFR in an experimental trial (Chiu et al. 1995). With IAP of 12 mmHg, urine output decreases and U-NAG obtained at the end of surgery is a third higher (Koivusalo 1997) compared with the present study. In humans there are no studies comparing effects of different IAPs on proximal tubular deterioration. In an experimental model in rats, IAP of 5 mmHg did not affect GFR, but with an IAP of 10 mmHg both GFR and urine output decreased (Lindström et al. 2003). Therefore, it remains to be established whether pneumoperitoneum with IAP 12 mmHg used in the present study induces any significant proximal tubular deterioration *per se*.

Micali and co-workers (1999) have evaluated the effects of pneumoperitoneum on proximal tubular integrity and they reported U-NAG/crea concentrations which were 5-fold higher than those found in the present study. There are some differences between the two studies that may explain the conflicting findings. In the Micali and co-workers (1999) study, some patients had renal diseases and malignancies or nephrotoxic antibiotic therapy, which may have affected U-NAG/crea levels.

Clonidine may compromise renal perfusion during pneumoperitoneum by decreasing heart rate, blood pressures and cardiac output (Kallio et al. 1990). The dose response of clonidine's hemodynamic effect is U-shaped: with both small, 1–2 μg kg^{-1}, and high, 6–8 μg kg^{-1}, doses rarely leading to hypotension in patients with clonidine. With moderate doses, as the dosage

4.5 μg kg^{-1} used in the present study, the blood pressure decrease is most significant (Lowenthal 1980, Eisenach et al. 1996). At a dose of 2 μg kg^{-1} intravenous clonidine does not decrease cardiac index below 2 L min^{-1} m^{-2} (Kallio et al. 1990), which is considered to be a critical limit for renal perfusion during laparoscopic surgery, but with a medium dose 3 μg kg^{-1} low cardiac index may ensue (Kallio et al. 1990). Therefore, in the present study, the renal blood flow and perfusion pressure may have been low during pneumoperitoneum causing the minor proximal tubular deterioration.

In summary, pneumoperitoneum and isoflurane anesthesia seems to cause a transient proximal tubular cellular deterioration and co-administration of clonidine may enhance the damage.

ANTIDIURETIC HORMONE

In the present study (study 2), clonidine prevented the effect of ADH on the renal collecting tubules permeability and urine output, as only 1/15 patients with clonidine compared to 8/15 controls developed oliguria, urine output ≤ 0.5 mL kg h^{-1}, which is the commonly accepted limit (Bellomo et al. 2004) for a risk of renal deterioration. Similar diuretic effects of clonidine have also been reported by other groups (Gellai and Edwards 1988, Hamaya et al. 1994).

PLASMA RENIN ACTIVITY

Plasma RA increases during laparoscopy (Joris et al. 1998). In the present study (study 2), clonidine premedication diminished significantly the increase in P-RA in patients having pneumoperitoneum for laparoscopic cholecystectomy. Two experimental studies (Reid et al. 1975, Nolan and Reid 1978) and a human study (Lenaerts et al. 2004) have shown a similar effect of clonidine on P-RA.

Increased P-RA induces a production of angiotensin II, which is a potent vasoconstrictor. The released angiotensin II and catecholamines, particularly noradrenaline mediate the vasoconstriction and increased blood pressure during pneumoperitoneum (Joris et al. 1998). In study 2, a positive correlation was noted with mean arterial pressure and P-RA supporting earlier studies (Reid et al. 1975). The serum catecholamine concentrations are lower in clonidine-treated patients than in patients not receiving clonidine (Quintin et al. 1991, Joris et al. 1998). However, in the present study, S-catecholamines were not measured, and thus, the possible relationship between catecholamines and increased blood pressures and heart rate noted in the present study remains unclear.

In summary, clonidine administration before pneumoperitoneum induces minor and transient proximal tubular cellular damage during laparoscopic cholecystectomy. However, the effects in P-RA, urine output, blood pressure, and heart rate after clonidine administration were confirmed.

FLUORIDE METABOLISM

EFFECT OF SMOKING ON THE METABOLISM OF ENFLURANE AND SEVOFLURANE

In the present study (study 3, 4) the effect of tobacco smoke on the biodegradation of enflurane and sevoflurane was different. Smoking induced enflurane metabolism, but no such effect was noted with sevoflurane. In the smokers, enflurane was metabolized to the same extent as the $AUCF_{0-24}$ was identical than in the smokers with sevoflurane. On the contrary, in the non-smokers F^- production from enflurane, serum $AUCF_{0-24}$ 198 μmol h L^{-1}, was one third less than in the non-smokers with sevoflurane, serum $AUCF_{0-24}$ 317 μmol h L^{-1}.

There were two factors, which may have affected the biodegradation of enflurane and sevoflurane (Study 3, 4). The activity of CYP 450 isoenzymes varies between individuals (Piao et al. 2003) and it is known that in patients with low activity of CYP 2E1 S-F^- remains at moderate concentration after sevoflurane anesthesia, while in the patients with high CYP 2E1 activity S-F^- increases significantly (Wandel et al. 1997). In the present study the CYP activity was not tested, but an uneven distribution of activity between the non-smokers and the smokers seems unlikely. Propofol was used for anesthesia induction and for maintenance after inhalation anesthesia. Propofol inhibits the CYP 2E1 (Wandel et al. 1997, Lejus et al. 2002), and thus propofol infusion may have decreased metabolism of sevoflurane and release of F^-. However, the amount of propofol used in the present trial was similar across the study groups (studies 3 and 4) making them comparable with each other. Whether propofol supplementation could protect patient at risk from renal failure during sevoflurane anesthesia remains unknown and needs further studies.

In summary, cigarette smoking increases release of F^- in patients with enflurane anesthesia but does not affect F^- production with sevoflurane anesthesia.

EFFECT OF ENFLURANE AND SEVOFLURANE ON GLOMERULAR FUNCTION

The inhalation anesthetic agents and their metabolite, F^-, induce renal deterioration. Based on experiences with methoxyflurane, the renal toxic threshold of S-F^- was suggested to be 50 μmol L^{-1} (Taves et al. 1970, Cousins and Mazze 1973). However, decreased crea clearance and defects in renal concentrating ability has been reported with lower S-F^-, 34 μmol L^{-1}, after 9.6 MAC-hour enflurane anesthesia in volunteers (Mazze et al. 1977). Therefore, it seems that not only the maximum S-F^-, but also the extent (concentration x time) of F^- exposure is important. This is supported by the literature that indicates that both the AUCF and the amount of intrarenal metabolism of inhalation anesthetic may be determinants in renal toxicity (Kharasch et al. 1995b, Malan 1995). In the case of methoxyflurane, the duration of F^- exposure is prolonged because methoxyflurane is highly tissue soluble and released slowly for metabolism. Due to the high tissue solubility and high proportion of metabolism (40–75%), S-F^- remains elevated for four to six days after the methoxyflurane anesthesia (Kharasch 1995). Methoxyflurane is metabolized actively in the kidneys and the rate of metabolism is four times higher than that of sevoflurane (Kharasch et al. 1995b).

Serum tumor associated trypsin inhibitor

In the present study, lower S-F^- than the previously supposed limit of 50 μmol L^{-1} for tubular toxicity (Cousins and Mazze 1973) caused glomerular dysfunction measured with S-TATI (study 4). After enflurane anesthesia, 4/33 patients and after sevoflurane anesthesia, 11/50 patients developed high S-TATI (over 3 nmol L^{-1}). In the sevoflurane study, 5/11 patients with pathological S-TATI had S-F^- 40 μmol L^{-1} or above.

Some patients developed glomerular dysfunction after gynecological surgery with one MAC-hour sevoflurane anesthesia (study 4) but GFR remained stable in all study patient after breast surgery with 3.3 MAC-hours sevoflurane anesthesia (study 1). Two factors may explain differences in GFR. Firstly, hydration during the operation day was slightly less in the patients undergoing gynecological surgery, 50 mL kg^{-1}, than in patients undergoing breast surgery, 65 mL kg^{-1}. Secondly, different methods of measuring GFR were used; S-TATI was used in the patients undergoing gynecological surgery and S-cystatin C and -β2-MG in the patients undergoing breast surgery. Serum cystatin C is a reliable and sensitive marker of mild glomerular dysfunction (Wasén 2004), but S-TATI may not be as appropriate marker of GFR in gynecological patients because S-TATI may increase in the patients with e.g. ovarian tumors and pelvic infections without any disturbance in renal function (Paavonen et al. 1989, Stenmann 2002). In the present study, the five patients with high S-TATI and S-F^- did not have known confounding factor for increased S-TATI.

In summary, S-F^- of 40 μmol L^{-1} or above, which is lower than previously suggested 50 μmol L^{-1}, may be associated with glomerular dysfunction after sevoflurane anesthesia. Sufficient fluid therapy may prevent glomerular dysfunction in connection with sevoflurane anesthesia.

PROXIMAL TUBULUS

In the present study (studies 3, 4) the proximal tubular effect of F^- was assessed with U-β2-MG. After enflurane anesthesia U-β2-MG remained at low concentrations but after sevoflurane anesthesia U-β2-MG increased in two patients, both had a high S-F^-, 40 and 71 μmol L^{-1}.

Two studies report elevated U-β2-MG after sevoflurane anesthesia. A transient increase in U-β2-MG is seen after two repeated 4 MAC-hour sevoflurane anesthesia within 30 to 90 days (Nishiyama et al. 1998). In a study with patients with moderate renal insufficiency, U-β2-MG increased after sevoflurane anesthesia from second postoperative day and returned to the baseline six days after surgery (Tsukamoto et al. 1996).

In summary, in addition to glomerular dysfunction also a transient tubular dysfunction may occur at S-F^- of 40 μmol L^{-1} or above, which is lower than the previously suggested renal toxic concentration of 50 μmol L^{-1} (Cousins and Mazze 1973).

SURGERY

PNEUMOPERITONEUM AND TUBULAR FUNCTION

In the present study (study 2), pneumoperitoneum with IAP of 12 mmHg did not induce significant proximal tubular deterioration, although a slight increase in U-NAG/crea was noted

during pneumoperitoneum. However, the changes in U-NAG/crea were transient and the increased concentrations returned to the baseline the first hours after pneumoperitoneum. Glomerular function, assessed with S-crea, remained stable during the operation and 24-hour follow-up.

Renal GFR and urine output decreases during pneumoperitoneum and this impairment may last hours after prolonged pneumoperitoneum (Chiu et al. 1995). Increased IAP causes deterioration in renal perfusion pressure (Harman et al. 1982), and elevated renal venous pressure is considered one of the mechanisms for the renal impairment (Doty et al. 2000). In the present study, renal venous pressure was not measured.

The type of pneumoperitoneum seems to affect NAG/crea response during laparoscopy. Higher U-NAG/crea is measured in patients undergoing laparoscopic surgery operated with conventional pneumoperitoneum, IAP of 12-13 mmHg with room temperature CO_2, than with abdominal wall retractor without CO_2 insufflation (Koivusalo 1997).

In summary, pneumoperitoneum with clonidine medication added may cause urine release of proximal tubular cellular deterioration biomarkers. However, in the present study the increase of these biomarkers was minor and returned to baseline shortly after surgery. On the contrary, glomerular function is preserved.

MAIN LIMITATIONS OF THE STUDY

STUDY GROUP SIZE

The study group size in the first three studies was small. In the 1990s when the first trials were conducted it was common to use this size or even smaller study populations when renal effects of inhalation anesthesia were investigated with sensitive renal biomarkers (Higuchi et al. 1993, Bito and Ikeda 1994, Higuchi et al. 1995, Bito et al. 1997, Eger et al. 1997, Ebert et al. 1998a). Based on results of the present study, it is estimated that at least 25 to 30 patients per group is necessary in these kinds of studies to be able to make any meaningful clinical conclusions.

TRADITIONAL AND NOVEL MARKERS OF RENAL FUNCTION

In the present study, sensitive biomarkers of renal cellular integrity and function were used. The use of novel renal markers has been criticized and their clinical meaning has been questioned (Mazze et al. 2000). The criticism by Mazze and co-workers (2000) is based on a meta-analysis of 22 clinical trials in 3400 patients with sevoflurane, propofol, isoflurane or enflurane that shows no renal deterioration measured with S-crea and blood urea nitrogen. However, S-crea and blood urea nitrogen concentrations are considered insensitive markers of renal integrity and function (Charlson et al. 1989, Kellen et al. 1994).

MEASUREMENT OF GLOMERULAR FUNCTION

Serum crea, S-cystatin C, S-β2-MG and S-TATI are used to assess GFR, but each of them has limitations. Serum crea is insensitive to small and rapid changes in renal function (Charlson et

al. 1989, Kellen et al. 1994). Serum cystatin C, -β2-MG and -TATI are more sensitive endogenous markers of glomerular function and they correlate well with crea clearance, (Harmoinen 2001, Tramonti et al. 1997). However, when the renal threshold for reabsorption of β2-MG and TATI is exceeded (for S-β2-MG > 6000 μg L^{-1}) they are excreted into the primary urine. Intact proximal tubules reabsorb and degrade U-β2-MG and -TATI, but deteriorated cells release these markers into urine.

TUMOR-ASSOCIATED TRYPSIN INHIBITOR AND URINE β2-MICROGLOBULIN

Pelvic infections and ovarian tumors present a confounding factor when assessing renal glomerular function with TATI, as S-TATI may increase in both conditions (Stenmann 2002). In the present study (study 4), two non-smoking patients with high S-TATI had a pelvic infection.

There are some limitations in using U-β2-MG as an indicator of tubular damage. β2-microglobulin is synthesized in most nucleated cells and lymphocytes, and lymphoproliferative diseases like multiple myeloma (Schardijn and Statius van Eps 1987) may interfere renal diagnostics. β2-MG is degraded at body temperature and at a pH lower than six (Schardijn and Statius van Eps 1987), so acidic urine may lead to misleadingly low U-β2-MG levels. In the present study, U-pH was adjusted to 7.0 or higher according to laboratory instructions (Hospital district of Helsinki and Uusimaa, Huslab 2005).

α1-MICROGLOBULIN

In the present study, U-α1-MG/crea was an unreliable indicator of proximal tubular dysfunction in surgical patients because already at baseline 6/30 patients had U-α1-MG/crea higher than upper limit of normal, 0.7 g mol^{-1}, obtained from healthy volunteers not undergoing surgery (Teppo et al. 2000).

SAMPLING

In the present study (study 1–4), urine markers were collected from spot samples at predetermined times. It is common to measure urine biomarkers from 24-hour collection of urine and indexed to U-crea or urine volume (Higuchi et al. 1995, Teppo et al. 2000). However, spot samples normalized to U-crea are also used (Wellwood et al. 1975).

The correction of urine markers to U-crea is criticized because the secretion of crea changes according to the age of patients. The use of uncorrected markers may be better if the age of patients varies greatly. Correction of renal biomarkers to specific gravity of urine has also been suggested. However, the specific gravity correlates closely with U-crea and it is not considered better than correction to U-crea (Moriguchi et al. 2003). In the present study the age range was large, from 20 to 64 years, so the uncorrected values of renal markers are also presented, but the conclusions of the results were made from urine biomarkers normalized to U-crea.

CLONIDINE AND CARDIAC PERFORMANCE

Impaired cardiac performance is one of the main risk factor for ARF (Wilson and Aronson 2001). Clonidine and pneumoperitoneum both reduce cardiac output (Kallio et al. 1990, Hirvonen et al. 1997). In the present study, cardiac output was not measured, but the patients were kept normovolemic (median central venous pressure 7 mmHg) by the i.v. hydration, so that blood pressure was adequate for renal perfusion and diuresis was maintained.

DRUG ADMINISTRATION

Administration routes and sites may have a major impact on drug pharmacokinetics. Especially peak concentration, time to peak concentration and elimination half-life may be affected by administration site (Grabinski et al. 1983).

Ketorolac and clonidine were administered intramuscularly, although the site of the injection was not standardized. Main sites were in the area of the deltoid or gluteus muscle. A previous study shows more accurate absorption from the deltoid muscle than from the gluteus muscle (Kentala et al. 1998). Pharmacokinetic parameters of ketorolac or clonidine were not measured in the present study.

CLINICAL ASPECTS

Increased mortality is related to ARF in connection with surgery (Chertow et al. 1998). In high risk patients, accurate and reliable monitoring of renal function is important and may help to prevent permanent renal failure. Even minor insults, if they occur repeatedly, may cause cumulative damage to renal integrity and function (Ronco and Flahault 1994). Therefore, specific and sensitive biomarkers are needed for early detection of harmful insults.

Various noxious stimuli may harm anatomically and physiologically specific entities of the kidneys. The new sensitive renal biomarkers may offer diagnostic accuracy for detection of function and site specific disturbances and cellular damage. However, the validity and reliability as well as the sensitivity and specificity of these biomarkers in surgical patients is to be established.

MEASURING OF RENAL FUNCTION

Renal function is commonly measured by S-crea and S-urea, but these markers detect only one third of patients with renal dysfunction (Charlson et al. 1989, Kellen et al. 1994). The simple and accurate biomarker S-cystatin C may be feasible in estimating GFR (Wasén 2004). Cystatin C is able to monitor incipient and age- and disease-related GFR impairment better than S-crea and calculated formulas (Wasén 2004).

INTERACTION OF NSAIDS AND SEVOFLURANE DURING SURGERY

The traditional NSAIDs, e.g. ketorolac, are used routinely as a part of multimodal analgesia to treat postoperative pain after surgery and this approach has been proven rather safe to the kidneys (Forrest et al. 2002). According to the present study, the combination of ketorolac and moderate length sevoflurane anesthesia, 3.3 MAC-hour, is safe considering renal function, although minor transient increase in proximal tubular markers were noted. However, preoperative administration of ketorolac increased bleeding and need of transfusions during breast surgery.

CLONIDINE AND RENAL FUNCTION

Clonidine prevented activation of RAAS during cholecystectomy with CO_2-pneumoperitoneum. Clonidine prevented the effect of ADH on collecting tubules so that diuresis increased. Slight increase in U-NAG/crea after clonidine as a sign of proximal tubular impairment was noted during pneumoperitoneum, although clinically significant renal deterioration was not seen in any of the patients. Clonidine during pneumoperitoneum may be useful to inhibit the stress responses and to maintain urine output, although the use of clonidine should be further evaluated because of the minor proximal tubular deterioration that may result.

THE EFFECT OF SMOKING ON METABOLISM OF ENFLURANE AND SEVOFLURANE

Various chemicals in cigarette smoke both inhibit and induce the CYP 450 enzyme. Production of toxic metabolites like F^- may increase in smokers during inhalation anesthesia. Smoking did not affect the metabolism of sevoflurane but did increase enflurane's metabolism. Prolonged enflurane anesthesia in smokers may increase S-F^- to nephrotoxic concentrations. Serum F^- 40 μmol L^{-1} or above was associated with glomerular impairment after sevoflurane anesthesia. However, the changes in glomerular and tubular function after short sevoflurane exposure were transient.

In summary, a nonselective NSAID may be used relatively safely in connection to 3.3 MAC-hour sevoflurane anesthesia, however, minor proximal tubular deterioration may be noted with ketorolac. The first dose should be given after the surgery in order to preserve hemostasis during the procedure. Clonidine prevents the effect of ADH on renal tubuli, but causes a minor and transient proximal tubular deterioration during laparoscopic cholecystectomy with conventional pneumoperitoneum. Smoking does not affect the metabolism of sevoflurane but increases the production of inorganic fluoride after enflurane anesthesia. Glomerular dysfunction and proximal tubular deterioration are noted after 1 MAC hour sevoflurane anesthesia in patients with S-F^- 40 μmol L^{-1} or higher, which is lower than the previously assumed renal toxic threshold of 50 μmol L^{-1}. However, the changes in glomerular and tubular function after short sevoflurane exposure are transient.

CONCLUSIONS

1. As indicated by novel biomarkers perioperative ketorolac, 90 mg in 24 hours, does not affect renal function, but may induce minor proximal tubular deterioration in patients having a 3.3 MAC-hour sevoflurane anesthesia.

2. After one MAC-hour sevoflurane anesthesia patients with $S\text{-}F^- \geq 40$ μmol L^{-1} and $AUCF_{0\text{-}24} \geq 500$ μmol h L^{-1} are at a risk to develop a glomerular dysfunction and a proximal tubular deterioration.

3. Smoking does not affect the metabolism of sevoflurane but after one MAC-hour enflurane anesthesia $S\text{-}F^-$ is significantly higher in smoking patients than in non-smokers.

4. Preoperative clonidine 4.5 μg kg^{-1} does not affect renal glomerular function but may cause a transient proximal tubular cellular damage in patients with pneumoperitoneum.

5. Further studies are warranted to establish the reference values for the novel biomarkers of renal function and cellular integrity in surgical patients having anesthesia.

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